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* For the purpose of this prize, molecular biology is defined as "that part of biology which attempts to interpret biological events in terms of the physico-chemical properties of molecules in a cell".

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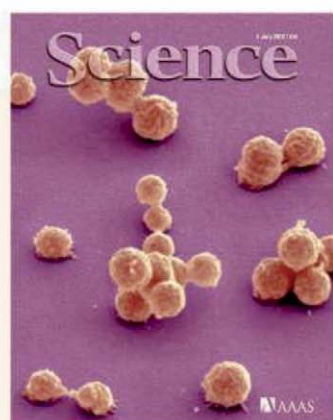
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COVER

Electron micrograph of *Mycoplasma mycoides* JCVI-syn1.0 cells (magnification ~25,000 $\times$). These cells were produced following transplantation of a 1.08-megabase pair synthetic *M. mycoides* genome into *M. capricolum* recipient cells. The cells are controlled by the synthetic genome, exhibit the expected phenotypic properties, and are capable of self-replication, thus providing proof of principle for the production of cells from digitized sequence information. See page 52.

Photo: Thomas Deerinck and Mark Ellisman, National Center for Microscopy and Imaging Research, University of California at San Diego

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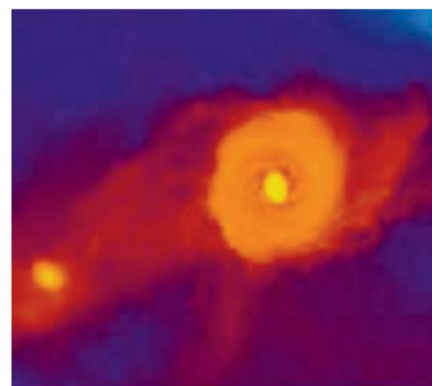
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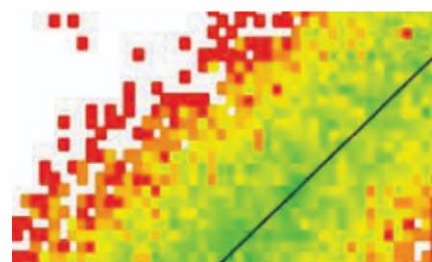
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T. Okiyonedo et al.

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Genetic Signatures of Exceptional Longevity in Humans

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10.1126/science.1190532

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RESEARCH ARTICLE: Serotonergic Neurons Mediate Dyskinesia Side Effects in Parkinson's Patients with Neural Transplants

M. Politis et al.

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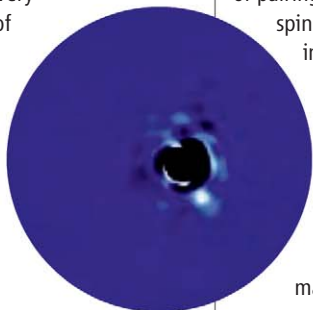
Although recent research has established the remarkable ways in which cognitive processing can occur without our being aware of it—for instance, casual exposure to retiree-related words, such as “elderly,” induces us to walk more slowly—behavior that is directed toward goals still seems to be the product of conscious thought. **Custers and Aarts** (p. 47) review a set of findings that point toward the possibility that goals may, in fact, also be vulnerable to manipulation via avenues of which we remain blissfully unaware. They place these results within a framework that reveals how thoroughly unconscious processes permeate our everyday activities.

Let There Be Life

The DNA sequence information from thousands of genomes is stored digitally as ones and zeros in computer memory. Now, **Gibson et al.** (p. 52, published online 20 May; see the cover; see the Policy Forum by **Cho and Relman**) have brought together technologies from the past 15 years to start from digital information on the genome of *Mycoplasma mycoides* to chemically synthesize the genomic DNA as segments that could then be assembled in yeast and transplanted into the cytoplasm of another organism. A number of methods were also incorporated to facilitate testing and error correction of the synthetic genome segments. The transplanted genome became established in the recipient cell, replacing the recipient genome, which was lost from the cell. The reconstituted cells were able to replicate and form colonies, providing a proof-of-principle for future developments in synthetic biology.

A Planet Is Born

The 10-million-year-old star β Pictoris, has long been suspected to host a planet. Through images obtained with the Very Large Telescope, an array of four telescopes located in Chile, **Lagrange et al.** (p. 57, published online 10 June) now confirm the presence of a young, giant planet, β Pictoris b, orbiting within the dusty disk that surrounds the star. β Pictoris b orbits closer to its star than Uranus and Neptune do to the Sun in our solar system. This orbital separation is consistent



One Two T

T cells develop in the thymus, where they proceed through several developmental stages, losing alternative lineage potential as they progress. The molecular regulation of this developmental process, however, is not fully understood (see the Perspective by Di Santo). **P. Li et al.** (p. 85, published online 10 June), **L. Li et al.** (p. 89), and **Ikawa et al.** (p. 93) now identify expression of the zinc finger transcription factor *Bcl11b* as the earliest checkpoint in T cell development in mice. Genetic deletion of *Bcl11b* in developing T cells inhibited commitment to the T cell lineage. Under conditions that should have stimulated T lineage differentiation, *Bcl11b*-deficient T cell progenitors failed to up-regulate genes associated with lineage-committed T cells and maintained stem cell- and progenitor cell-associated gene expression. In both developing and committed T cells, loss of *Bcl11b* resulted in the generation of cells that resembled natural killer (NK) cells in both phenotype and function. These NK-like cells could be expanded easily in vitro and possessed antitumor cytotoxicity, but they did not exhibit cytotoxicity against normal cells and were not tumorigenic. Because T cells are much easier to obtain from human patients than NK cells, deletion of *Bcl11b* in T cells may thus provide a source of easy-to-grow NK cells for cell-based antitumor therapies.

with the in situ formation of the planet via a core accretion mechanism. Thus, giant planets can form within a stellar dust disk in only a few million years.

Maintaining the Supercurrent

When a superconductor is placed in contact with a ferromagnet, the antiparallel spin pairs that form the supercurrent are expected to be broken almost immediately upon entering the ferromagnet, which tends to orient spins parallel to each other. If the supercurrent survives for more than a few nanometers, it is assumed that a change of pairing symmetry has taken place, with the spin-singlet pairs having been converted into spin-triplets. Magnetic inhomogeneity at the superconductor-ferromagnet interface is thought to account for this change. **Robinson et al.** (p. 59, published online 10 June) have now been able to observe long-ranged supercurrents in a symmetric junction consisting of a superconductor, a conical magnet, and a ferromagnet. The conical magnet layer provided the required inhomogeneity, and varying its thickness enabled control over the magnitude of the current.

Quantum Anomalous Hall Effect

In addition to the Hall effect, which appears as a voltage change in conductors in response to an external magnetic field, ferromagnets exhibit the anomalous Hall effect, which is often proportional to their magnetization and independent of the presence of the magnetic field. This effect, first observed more than a century ago, has not been realized in its quantized form. **Yu et al.** (p. 61, published online 3 June) propose a realization of a quantum anomalous Hall system by magnetically doping thin films of three-dimensional topological insulators and calculate the effects of various dopants and film thicknesses. The resulting insulators are predicted to have long-range ferromagnetic order, potentially joining dilute magnetic semiconductors as candidates for spintronic applications.

Filling a Cavity

Unlike liquid ammonia, water cannot sustain a steady concentration of isolated electrons. Nonetheless, high-energy irradiation can introduce a small number of free charges that engage in potent reductive chemistry and have clear spectroscopic signatures. The manner in which water solubilizes these hydrated electrons

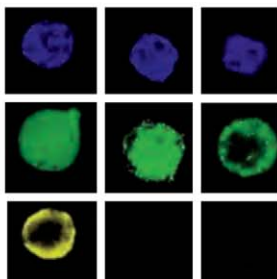


has remained uncertain, but the general consensus has been that repulsive interactions drive the nearest water molecules away, leaving the electron in a nearly spherical empty cavity. **Larsen *et al.*** (p. 65; see the Perspective by **Jordan and Johnson**) upend this consensus with simulations based on a more thorough potential function for modeling the competing attractions and repulsions between the electron and surrounding water. The calculations suggest that the hydrated electron actually draws water in, occupying a region denser than the pure bulk liquid. The model reproduces experimental spectral and dynamic observations as effectively as, and in some cases better than, the cavity framework.



Erasing Markers

Epigenetic reprogramming of the mammalian genome, which involves the removal and replacement of the various regulatory epigenetic marks such as DNA methylation, occurs during germ cell differentiation and during early zygotic development. This process is also critical during the experimental generation of stem cells, but the factors and pathways that control epigenetic reprogramming are not well understood. **Hajkova *et al.*** (p. 78) investigated the erasure of DNA methylation during germ cell differentiation and during early zygotic development in the developing mouse and found that factors involved in the base excision repair (BER) pathway, which helps repair damaged DNA, were involved. Furthermore, inhibitors of BER resulted in the retention of DNA methylation in the zygote.



Early Rising Hydrogen

Formation of molecular hydrogen through electron-expelling collisions of H atoms and H⁻ anions is regarded as a key step in the cooling process that led to assembly of the first stars in the early universe. **Kreckel *et al.*** (p. 69; see the Perspective by **Bromm**) performed highly precise laboratory measurements of the rate of this reaction at a range of different energies. The study required construction of a dedicated apparatus to carefully tune the relative velocity of merged atom and ion beams. The data validated prior theoretically calculated reaction cross sections, which were then extended for use in cosmological models.

No Genetic Vertigo

Peoples living in high altitudes have adapted to their situation (see the Perspective by **Storz**). To identify gene regions that might have contributed to high-altitude adaptation in Tibetans, **Simonson *et al.*** (p. 72, published online 13 May) conducted a genome scan of nucleotide polymorphism comparing Tibetans, Han Chinese, and Japanese, while **Yi *et al.*** (p. 75) performed comparable analyses on the coding regions of all genes—their exomes. Both studies converged on a gene, *endothelial Per-Arnt-Sim domain protein 1* (also known as *hypoxia-inducible factor 2α*), which has been linked to the regulation of red blood cell production. Other genes identified that were potentially under selection included adult and fetal hemoglobin and two functional candidate loci that were correlated with low hemoglobin concentration in Tibetans. Future detailed functional studies will now be required to examine the mechanistic underpinnings of physiological adaptation to high altitudes.

Dodgy Repair

A double-strand break (DSB) in genomic DNA poses a serious threat to genome stability, and yet vertebrate cells may suffer 10 or more DSBs every time the genome is replicated. Pathways have thus evolved that can recognize and repair DSBs before they wreak havoc in the cell. **Hicks *et al.*** (p. 82) show that this repair can come at a price. DSBs were induced in the budding yeast genome. Repair of a break was accompanied by a massive increase in the rate of mutation in the vicinity of the break. The mutations generated displayed a specific “signature” that included the copying of divergent sequences from other chromosomes.



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Science Careers

From the journal *Science*





Farouk El-Baz served as Science Advisor to President Anwar Sadat of Egypt and is director of the Boston University Center for Remote Sensing and a member of the U.S. National Academy of Engineering.

Science Attachés in Embassies

THE UNITED STATES' INTENTION TO INCREASE INTERNATIONAL COOPERATION IN SCIENCE AND technology (S&T) is welcome news. Such efforts would be particularly effective in the Muslim world, where countries are in need of the economic development that would result from improvements in their S&T sectors. Last year, President Obama appointed three Science Envoys to Muslim-majority countries: Bruce Alberts, former president of the U.S. National Academy of Sciences (and current Editor-in-Chief of *Science*); Ahmed Zewail, Nobel Laureate and professor at the California Institute of Technology; and Elias Zerhouni, former director of the U.S. National Institutes of Health. The appointment of several more Science Envoys is expected soon. However, this promising new initiative will require committed institutional support, which can best be provided by the appointment of Science (and Technology) Attachés in the relevant U.S. embassies.

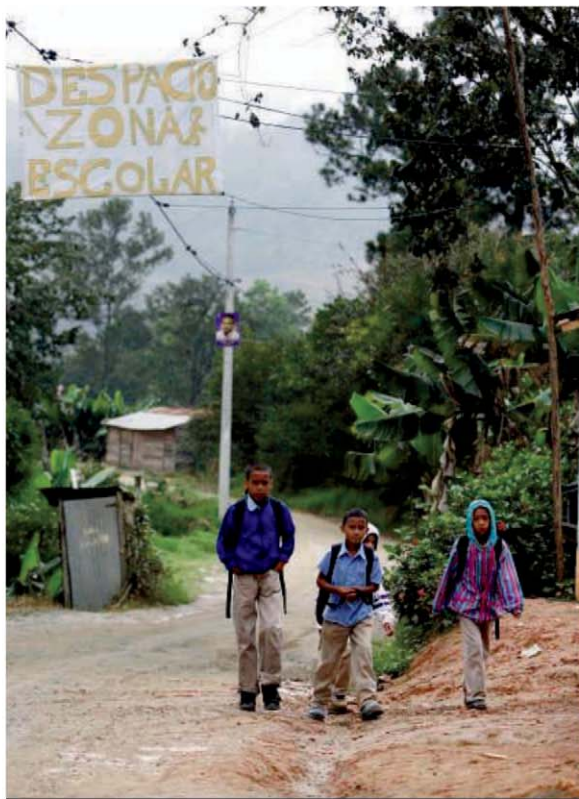
Many U.S. embassies had such attachés in the past. During my 10 years (starting in 1973) at the U.S. Smithsonian Institution, the services of Science Attachés were a great advantage. In Moscow, such an attaché assisted our delegation throughout discussions about joint production of a chart of lunar surface features, as well as Earth photography on the Apollo-Soyuz Test Project of 1975. In Egypt, a Science Attaché facilitated my team's work in studying its vast deserts, including securing field permits and shipping samples to the United States. And in India, the Science Attaché paved the way for our research in the Rajasthan Desert. These individuals also maintained contact with many researchers and institutions, leaving open the doors for future cooperative projects. The type of assistance that I received in these countries required a person with extensive science and engineering knowledge. Unfortunately, the Science Attaché position was abolished two decades ago, but it is needed more than ever today to achieve the Obama Administration's objectives.

By their nature, cooperative S&T programs are long-term, requiring attention over extended periods of time. Only embassy personnel can meet this need effectively, following up on the contacts made by science envoys and other U.S. visitors. A Science Attaché can also assess the S&T capabilities and needs of a country by collecting the opinions of decision-makers and local professionals. This information can help to guide the U.S. Administration on the best short- and long-term strategies, forming links for local counterparts to the most appropriate U.S. institutions for joint research, education, and development activities.

The appointment of Science Attachés in U.S. embassies would highlight what many countries greatly respect about the United States: its first-rate S&T. How might the outstanding people required for such posts be selected, trained, and supported? The U.S. Department of State (DOS) should not be expected to create this program alone. The DOS could recruit the National Academies to recommend the best individuals for specific posts, and other agencies with successful attaché programs can provide a proven framework. The U.S. Department of Agriculture supports scientific experts inside embassies. Following this model, other agencies with technical expertise, including the National Science Foundation, National Institute for Standards and Technology, and National Oceanic and Atmospheric Administration, could provide staff for a Science Attaché program. In each case, a subject-matter expert would be placed within an embassy, fully integrated into the embassy's country team. Foreign service staff dedicated to diplomacy issues related to the environment, science, technology, and health are also needed. Ironically, this position is often absent from embassies, even though it is a sought-after career path. All such individuals, supported by the Science and Technology Advisor to the U.S. Secretary of State and the Bureau of Oceans, Environment, and Science, could also benefit the director of the White House Office of S&T Policy on international matters. By working as a team, Science Attachés and Science Envoys should produce striking results, thereby securing the longevity of a new science diplomacy.

— Farouk El-Baz





ECONOMICS

Educating the Consumers

Many efforts to improve education aim to increase the supplies of teachers and schools; in some cases, supply may outstrip demand, which is thought to be driven in part by long-term earnings that accrue with increasing education. However, many families, especially those in developing countries, may lack complete information about long-term returns, and misperception of the economic benefits could influence demand. In the Dominican Republic, for example, large declines in enrollment are seen after the eighth grade, which marks the final year of compulsory education. Jensen asked 2250 of those eighth-grade boys to estimate the earnings of adult men who had completed primary school only, and also of men who had spent 4 more years to complete secondary school. The estimated benefit for completing secondary school was only one-fourth as large as the actual benefit. A randomly chosen subset of boys was then given accurate information about the enhanced earnings. Four years after the initial survey, those boys had completed roughly 0.2 more years of schooling as compared to boys who were not given earnings information. Although some educational interventions offer near-term financial rewards directly to students, for example in return for high test scores or reading lots of books, a supply of earnings information may be an inexpensive way to promote more years of schooling. — BW

Quart. J. Econ. **125**, 515 (2010).

NEUROSCIENCE

Self-Assembled Circuits

The mammalian brain develops as growth factors and transcription factors define fields of morphogenesis, with some types of neurons migrating outward from deep generative zones and other types of neurons migrating along the surface from distant generative zones. The result might be compared to a geopolitical map supported by an infrastructure of shipping, communication, and regulatory networks. Zhou *et al.* used a mouse mutant in which the neocortex had been disconnected from the rest of the brain in order to analyze the development of the surface map. In normal mice, a few weeks of postnatal development complete the brain's organization; the mutant mice survive during this phase but die at about 3 weeks of age. During these weeks, the mutant mice, despite having disconnected brains, display a variety of behaviors: eating, drinking, walking, and swimming. Some aspects, such as circadian rhythms of activity, are noticeably disrupted. Interneurons are present at normal densities, although reduced numbers reflect the generalized atrophy of their brains. Projection neurons seem particularly susceptible to attrition,

and layer 4 of the cortex seems particularly dependent on its connections. Otherwise, the disconnected mouse brain shows a considerable ability to organize itself. — PJH

J. Neurosci. **30**, 7928 (2010).

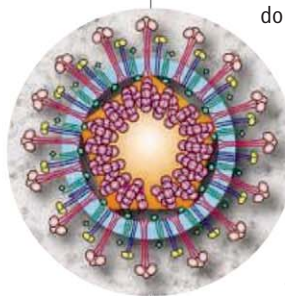
CELL BIOLOGY

Exploiting an Exploiter

Viruses have evolved diverse mechanisms to take control of the host cells in which they replicate. All plus-stranded RNA viruses, such as hepatitis A and poliovirus, use host cell membranes for replication in the cytoplasm. Coronaviruses such as SARS and mouse hepatitis virus (MHV), which infect the mammalian gastrointestinal and respiratory tracts, induce the formation of double membrane vesicles; the cellular origin of these membranes has been unclear. Reggiori *et al.* find that MHV accumulates cellular membranes by hijacking part of a protein degradation pathway known as ERAD, which is associated with the endoplasmic reticulum. As a quality-control step in protein degradation, the chaperone protein EDEM1 is sequestered

in small vesicles away from the endoplasmic reticulum to distinct cellular compartments. MHV exploits this event to obtain endoplasmic reticulum-derived membranes for its own use. Both EDEM1 and the autophagy-associated protein LC3 associated with virus-induced double membrane vesicles, and down-regulation of LC3 protected cells from MHV infection. By identifying the mechanism whereby this coronavirus recruits host membranes for replication, the authors have identified potential therapeutic targets, which may also be applicable to other viruses in this family. — HP

Cell Host Microbe **7**, 500 (2010).



NEUROSCIENCE

No Slacking Off

Characteristics of the inherited mental disorder fragile X syndrome are deficiencies in learning, memory, and cognition. The condition results from the lack of fragile X mental retardation protein (FMRP), which binds to RNA and to cytosolic proteins involved in protein synthesis.

Brown *et al.* have found that FMRP binds to a third type of target: a membrane protein called Slack, which is a sodium-activated potassium channel. FMRP increased Slack channel activity in a *Xenopus* oocyte expression system and altered the pattern of electrical conductance, and sodium-dependent potassium channel conductance was reduced in cells isolated from the brain of mice lacking FMRP. These channels are known to maintain neuronal activity during high-frequency stimulation, and these findings point to a role for FMRP in sustaining a level and pattern of channel activity during repetitive neuronal stimulation. — LC

Nat. Neurosci. **13**, 10.1038/nn.2563 (2010).

GEOLOGY

Slow Slip in Depth

The largest earthquakes occur at subduction zones, which accommodate the convergence of two tectonic plates. Thanks to dense GPS and seismic networks along these zones, we have a more detailed, and now complicated, view of how this convergence occurs: Many subduction zones exhibit a phenomenon termed "slow slip." Gombert *et al.* provide an overview of this process in the Cascadia Subduction Zone, where it has been heavily studied. Here, the GPS data show that the westward motion of the North American plate reverses regularly over periods ranging from days to several weeks. Unusual small earthquake tremors at depths of 40 to 50 km along the subduction zone, a bit deeper than the area expected for major quakes, increase dramatically at this time and explain the lag in plate motion. Nearly 40 such events have been studied in the Cascadia subduction zone, and they also vary from north to south. The current model posits that the slow slip is related to fluid release by metamorphic reactions in the subduction zone, but implies that the plate interface at depth is weak and critically stressed. Thus understanding and monitoring slow slip may be critical for hazard assessment here and elsewhere. — BH

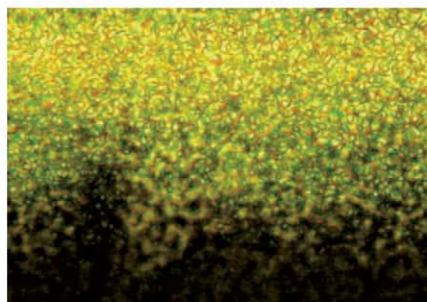
Geol. Soc. Am. Bull. **122**, 963 (2010).

CHEMISTRY

Stacked Platinum

Platinum is a remarkably versatile chemical catalyst. Recently, chemists have also begun to direct its coordination properties toward the assembly of complex supramolecular structures. Frischmann *et al.* constructed a family of tunable Schiff base proligands that were designed to self-assemble in head-to-tail fashion when coordinated to Pt²⁺. The proligands—substituted salicylaldehydes—could be generated in situ

or before metalation. Puckered Pt₄ macrocycles formed that would then stack in either single or alternating configurations, depending on the bulkiness of the peripheral R group, with the latter dominant as the R group increased in size. The authors were surprised to find that when R was 2-hexyldecyl, the columnar macrocycles formed lyotropic liquid crystalline



phases when concentrated in nonpolar organic solvents. The complexes showed poor solubility in cyclohexanone, but when cast from a solution using this solvent, they formed parallel columns that were spaced 4 nm apart and extended for hundreds of nanometers. These arrays thus offer a method to access nanotubes with stacked columns of Pt²⁺ ions. — MSL

J. Am. Chem. Soc. **132**, 7668 (2010).

CHEMISTRY

Oxidative Flow

Alcohol oxidation is a common step in the synthesis of pharmaceutical intermediates, and oxygen is in many ways the ideal reagent to induce it. The gas is plentiful and nontoxic, and so is the by-product, water, that it's shuffled into as the reaction ensues. Unfortunately, oxygen also has several serious drawbacks that have largely precluded its use in this context. In too pure a form, it can react violently with solvents and other peripheral organic fragments. At the same time, it is a competing challenge to keep enough of the gas mixed with the liquid reaction medium to inhibit catalyst decomposition. As a result, oxidations still often rely on expensive reagents and generate associated toxic by-products. Ye *et al.* now present a reactor design that makes substantial progress in surmounting these obstacles. They employ a dilute stream of 8% oxygen in nitrogen, so as to avoid combustion hazards, and they implement a continuously flowing reaction process to optimize mixing. Using several previously reported palladium-based catalysts, they demonstrate high alcohol oxidation yields across a diverse substrate pool on scales ranging from tens of grams to a kilogram. — JSY

Green Chem. **12**, 10.1039/c0gc00106f (2010).



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Big Whales, Tiny Numbers

Almost 200 years ago, whalers in the Gulf of Alaska could scarcely believe the numbers of North Pacific right whales (*Eubalaena japonica*) living there. Today, scientists are astonished because the numbers are so low. Genetic and photographic data reveal that between 30 and 54 of the behemoths remain, and only eight are female, according to a study in the 30 June issue of *Biology Letters*. "It is the world's smallest whale population for which we have estimates," says lead author Paul Wade, a whale biologist at the National Oceanographic and Atmospheric Administration's Alaska Fisheries Science Center in Seattle, Washington.

American whalers harpooned more than 23,000 North Pacific right whales in the 19th century. After the whales were given international protection in 1949, the Soviet Union hunted them illegally until none were seen in the region (*Science*, 29 May 2009, p. 1132). "They hit the recovering population [of about 400 whales] hard," Wade says. A few were rediscovered in 1996.

"The good news is that the population is large enough to be measured," says Andrew Read, a cetacean biologist at Duke University in Durham, North Carolina. So little is known about the whales, he says, "we still do not know where they breed."

Gruber Prizes

Gerald Fink of the Massachusetts Institute of Technology in Cambridge has snagged the Gruber Genetics Prize, announced Wednesday. Fink pioneered using living yeast cells to express genes from any organism. The Cosmology Prize goes to the California Institute of Technology's Charles Steidel, who is known for discovering early star-forming galaxies with a novel technique. The Neuroscience Prize goes to the National Institutes of Health's Robert Wurtz, whose groundbreaking physiological studies on monkeys helped elucidate vision processing. Each winner will receive \$500,000 and a gold medal from the Peter and Patricia Gruber Foundation, based in St. Thomas, U.S. Virgin Islands.

Saving an Evolutionary Icon

The Galápagos Islands are often called a "living laboratory of evolution." But they've also become a laboratory of conservation, as scientists scramble to save the islands' storied species from the ravages of human contact.

Galápagos's finches, a famous example of how adaptations to different food sources can lead to new species, have been particularly hard hit. Egg-eating ship rats and other ills have whittled down one species, the mangrove finch, to 100 birds, nearly all living on one stretch of beach on Isabela Island.

In mid-June, the Ecuadorian government announced that scientists had captured nine of the birds and moved them across the island to a new, largely rat-free habitat 25 kilometers away

in a bid to increase their numbers. Researchers have successfully reintroduced breeding iguanas and tortoises to islands where their numbers had dwindled, but this is the first relocation of a Galápagos bird.

"It's a calculated risk to take 10% of the population," says H. Glyn Young, a conservation biologist at Durrell Wildlife Conservation

Trust in the Channel Islands, which runs the project with the Galápagos National Park and the Charles Darwin Foundation.

Since the relocation in late May, only one of the finches has flown home. A field expedition this month will check on the rest. If the relocation succeeds, Young says his team has

"more-ambitious plans" to place mangrove finches in two other habitats. Also being eyed for relocation is the Floreana mockingbird, a species that Darwin cited as an inspiration for his theory of natural selection.



Art at World's End

Artist Xavier Cortada favors an unusual medium: polar ice. In 2006, during 2 weeks in Antarctica, he took scientists' samples from the West Antarctic Ice Sheet, mingled them with paint, and let them melt on paper. "It's a watercolor if you think about it," Cortada says, and "a precursor of horrors to come" with global warming.

In a performance piece at the South Pole, Cortada read 24 statements about climate change, collected from people around the world, over 24 shoes aligned with their longitudes. He also planted 51 flags 10 meters apart to mark how ice has flowed yearly from the pole since 1956, when humans opened a permanent base there, and buried an ice replica of a mangrove seedling under the pole itself. Cortada called the latter installation "150,000-Year Journey"—the time the ice will take to convey the



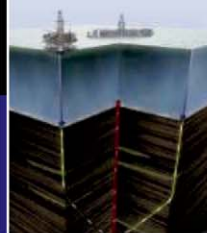
seedling to the continent's edge. "It's my way of ... making us realize how insignificant [human history] was next to geologic time scales," he says.

Artifacts, photos, and video from Cortada's projects appear in *North Pole/South Pole (90n/90s) Installations*, an exhibition that opened 25 June at the Miami Science Museum in Florida.



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to the oil spill

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How to drill
a killer well

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CHRONIC FATIGUE SYNDROME

Conflicting Papers on Hold as XMRV Frenzy Reaches New Heights

It was just a snippet of news, reported by an obscure journal in the Netherlands. And yet it lit up the Internet. Twitter was all atwitter, scientists' mailboxes on both sides of the Atlantic began filling up, and dozens of bloggers started jubilating. "It's happened. I cannot tell you all how this changes the world as we have known it for 25+ years," one patient wrote on her blog. "Now to work on the vindication part!"

The reason for all the excitement? Scientists at the U.S. National Institutes of Health (NIH) and the Food and Drug Administration (FDA) were reported to have confirmed the link, first published in *Science* last year, between a human retrovirus and the elusive condition called chronic fatigue syndrome (CFS). Earlier this year, three other groups reported being unable to replicate such a connection. That federal scientists now confirmed it was a huge mood-lifter for patients, many of whom are desperate to find a biological cause, and a cure, for their debilitating ailment.

But the story wasn't as simple as that. *Science* has learned that a paper describing the new findings, already accepted by the *Proceedings of the National Academy of Sciences* (PNAS), has been put on hold because it directly contradicts another as-yet-unpublished study by a third government agency, the U.S. Centers for Disease Control and Prevention (CDC). That paper, a retrovirus scientist says, has been submitted to *Retrovirology* and is also on hold; it fails to find a link between the xenotropic murine leukemia virus-related virus (XMRV) and CFS. The contradiction has caused "nervousness" both at PNAS and among senior officials within the Department of Health and Human Services, of which all three agencies are part, says one scientist with inside knowledge.

The remarkable impasse is the latest twist in a story that keeps arousing fierce passions. It started in 2009 when a group of researchers led by Judy Mikovits of the Whittemore Peterson Institute (WPI) for Neuro-Immune Disease in Reno, Nevada, reported in *Science*

finding traces of XMRV in peripheral blood mononuclear cells, a type of white blood cell, of 67% of CFS patients. By contrast, only 3.4% of healthy controls were found to harbor the virus. The team also showed that XMRV could infect human cells and concluded that the virus—which had previously been linked to prostate cancer—might play a role in causing CFS (*Science*, 23 October 2009, p. 585).

Many scientists were skeptical, however, and in May, *Science* published three Technical Comments that tried to poke holes in the study, along with a rebuttal by Mikovits and first author Francis Ruscetti of the National



Real-time results for XMRV

- stemcell4autism News in a nutshell: XMRV and GM Animals... <http://blog-the-scientist.com/2010/05/28/news-in-a-nutshell-4/>
about 1 hour ago via web
- NoFun Blog XMRV: ¿CAUSA O EFECTO DE LA INMUNODEPRESION DEL SÍNDROME DE FATIGA CRÓNICA? <http://tinyurl.com/236kl3l>
about 2 hours ago via web
- laikas wht do do if patients react emotional abt a @researchblogging-post & just write letters in response w/our reading urs? <http://bit.ly/az7cVZ>
about 3 hours ago via Twitter
- FIBRO360 The NIH voiced their support for preliminary research linking CFS to the XMRV. This may help with funding for research. <http://bit.ly/9FzJu6>
about 4 hours ago via web
- HEIRS_Health NIH Supports Findings of XMRV in Chronic Fatigue Syndrome <http://fb.me/DnUQCluf>
about 5 hours ago via Facebook
- CFSyndrome Further Evidence of an XMRV-Chronic Fatigue Syndrome Connection ... A report that a respected NIH expert supports... <http://bit.ly/b2qalz>
about 11 hours ago via twitterfeed
- overcome_ME_CFS Further Evidence of an XMRV-Chronic Fatigue Syndrome Connection ... <http://bit.ly/cQeVlc>
about 15 hours ago via twitterfeed
- overcome_ME_CFS BREAKING NEWS: FDA & NIH confirm WPI team's XMRV & CFS findings ... <http://bit.ly/d9ldTM>
about 18 hours ago via twitterfeed
- malitosabrina Further Evidence of an XMRV-Chronic Fatigue Syndrome Connect | Health: Further Evidence of an XMRV-Chronic Fatigue... <http://bit.ly/aGsell>
1 day ago via twitterfeed
- Diplead RT @fibroadrienne: NIH Supports Findings of XMRV in Chronic Fatigue Syndrome: <http://bit.ly/9Ujys4h>
1 day ago via Hootsuite

Going viral. News that federal scientists confirmed the virus link reported by Judy Mikovits (*inset*) sparked thousands of tweets.

Cancer Institute. By that time, two groups in the United Kingdom and one in the Netherlands had also published papers failing to find a link; in fact, they found little or no evidence of XMRV infection at all, either in patients or in healthy people. Three other groups, two from the United States and one from Europe, have also reported negative findings at meetings, says Kim McCleary, president of the Chronic Fatigue and Immune Dysfunction Syndrome Association of America, a patient advocacy group.

The FDA-NIH paper would offer fresh hope that Mikovits is on to something after all, but so far, details about the work are scant. *Ortho*, a Dutch magazine about nutrition and food supplements, last week issued a press release saying that Harvey Alter, a renowned virologist at NIH's Clinical Center, mentioned the study when he gave a talk at a blood safety meeting in the Croatian capital Zagreb in late May. In his Power-Point presentation, Alter wrote that the data in the 2009 study in *Science* "are extremely strong and likely true, despite the controversy." Another bullet point said: "We (FDA & NIH) have independently confirmed the Lombardi group findings." (WPI's Vincent Lombardi was the paper's first author.) But the presentation offered no detail beyond that tantalizing summary, and an NIH spokesperson says Alter is not available for comment.

Meanwhile, a group working with retrovirologist William Switzer at CDC, which has done an independent study, has held its cards closer to its chest. But *Science* talked to several scientists who say they have seen the data, and they are negative. Although it's not unprecedented for government scientists to be on opposite ends of a scientific debate, two contradictory press releases on a flash-point issue like CFS would look odd, scientists say. With publication deferred, "they want to find out what's going on first," says one researcher who says he has been briefed about the controversy.

For patients, the stakes are huge. A viral cause would offer a path to better understanding, prevention, and treatment. A paper published in *PLoS ONE* in April showed that three registered antiretroviral drugs can inhibit XMRV in the test tube, and although many scientists warn that it's pre-

CREDITS (TOP TO BOTTOM): SARAH KIEWEL/UNIVERSITY OF FLORIDA; 2010; TWITTER



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mature even to consider them as treatment options, some patients have started testing out different combinations anyway. (One of them is Jamie Deckoff-Jones, a physician from Santa Fe, New Mexico, who is blogging about her experiences and those of her daughter, also a patient.)

Patients have become a loud voice in the scientific debate as well—and it's taking its toll on scientists who don't support the XMRV hypothesis. "It's ghastly," says retrovirologist Myra McClure of Imperial College London, the lead author on one of the three published studies that came up

empty-handed. "I've had people writing me, and I quote, that I don't know my arse from my elbow, and that I should be fired." Four months after her first paper on CFS came out, McClure says it was also her last one. "Nothing on God's Earth could persuade me to do more research on CFS," she says. "I feel bad for the scientists, because it's true, we are a very angry community," says Wilhelmina Jenkins, a physicist living in Atlanta who has had CFS since 1983.

The potential involvement of XMRV in CFS is having other ramifications as well. Last week, the AABB, an international asso-

ciation of blood banks, recommended to its members that they discourage CFS patients from donating blood (<http://bit.ly/chronic-fatigue>). A special task force on XMRV conceded that the evidence was preliminary but decided it's "prudent" to err on the side of caution, says task force member Louis Katz, the medical director at the Mississippi Valley Regional Blood Center in Davenport, Iowa. "If [XMRV] turns out to be important," says Katz, "I don't want to be criticized for doing nothing when I could have done something."

—MARTIN ENSERINK

BHOPAL

India Launches New Probe of Cyanide Disaster

NEW DELHI—More than a quarter-century after the world's worst chemical disaster, the Indian government last week ordered remediation of the contaminated site in Bhopal and the creation of a research institute to study the toxic legacy.

The tragedy began on the night of 3 December 1984, when more than 40 tons of methyl isocyanate gas escaped from Union Carbide Corp.'s pesticide plant in Bhopal. Investigators learned that three safety systems, including a flare tower that could have burned off the leaking gas, were all switched off at the time of the accident. The gas killed 5295 people within days, and, according to local authorities, nearly 10,000 more people have subsequently died of complications from cyanide lesions.

The government has now instructed the Indian Council of Medical Research (ICMR) to set up a center in Bhopal to study respiratory diseases, cancers, genetic disorders, and birth defects. The decision is controversial because years of research have uncovered only acute effects of the gas. The new center is a case of "political expediency," charges Sushil Kumar Jain, a lung specialist here at Moolchand Hospital and lead author of many Bhopal studies.

The move comes in the wake of public outrage over verdicts handed down last month to seven Union Carbide executives, each of whom was sentenced to a maximum of 2 years in prison for negligence. The light sentences in the long-running case are a

"travesty of justice," claims Sunita Narain, director of the Centre for Science and Environment, a nonprofit here. Stung by widespread criticism, the government is looking to retry the accused on the far more serious charge of "culpable homicide."

Bhopal is already familiar turf for ICMR. In an exhaustive report on the accident in 2004, ICMR found that the gas inflicted acute injuries to eyes and lungs but did not trigger cancers or other "progressive" diseases. ICMR also found no evidence of genetic or congenital abnormalities. There is "no interest among researchers to study this tragedy anymore," contends ICMR Director General Vishwa Mohan Katoch. In 2008, he notes, a call for proposals for more Bhopal medical studies drew a mere three responses. Still, Katoch says, ICMR must comply with the order to set up a new center within 90 days.

Also raising hackles is a decision to transfer control of Bhopal Memorial Hospital and Research Centre, established after the accident, to the Department of Biotechnology (DBT) and the Department of Atomic Energy. "This is the limit of absurdity," says molecular biologist Pushpa M. Bhargava, chair of the Sambhavana Trust in Bhopal, a nonprofit body helping victims. "What experience does the Department of Biotechnology have in patient



Paying homage. D. K. Satpathy of Gandhi Medical College and colleagues conducted over 2500 postmortems in 3 days in 1984.

care?" According to DBT Secretary Maharaj Kishan Bhan, the hospital will become a base for environmental biomedical research. The government has also allocated \$77 million to dismantle the plant and for "complete" restoration of the grounds by the end of 2012.

Earlier this year, the National Environmental Engineering Research Institute (NEERI) in Nagpur found no contamination of groundwater in five wells dug at least 25 meters deep at the site. What saved Bhopal from a bigger catastrophe, says NEERI Acting Director Tapan K. Chakrabarti, is a 17-meter-thick layer of clay that has blocked pollutants from leaching into the water table—a silver lining, perhaps, to Bhopal's gloomy story.

—PALLAVA BAGLA

CREDIT: PALLAVA BAGLA

CONDENSED-MATTER PHYSICS

Evidence for Free-Flowing Supersolid Slipping Away?

Six years ago, frigid solid helium set the world of condensed-matter physics afire when one team found that at temperatures near absolute zero the stuff appears to flow like a liquid without any viscosity (*Science*, 1 July 2005, p. 38). Physicists have since debated how such “supersolidity” might come about, or whether it even exists. Now, an experiment calls into question the first, best evidence for supersolidity. What physicists took as a sign of resistance-free flow at very low temperatures is in fact a manifestation of an odd softening of the solid at slightly higher temperatures, says John Reppy of Cornell University.

If the result can be reconciled with previous experiments, “then I think John’s conclusion that what we’re seeing is not low-temperature supersolidity ... is kind of hard to escape,” says John Beamish, an experimenter at the University of Alberta in Edmonton, Canada. But others say that supersolidity

They set a little can of the material twisting atop a small metal shaft in a “torsional oscillator.” When the temperature dropped below about 0.2 kelvin, the frequency of the twisting shot up, suggesting that some helium atoms had let go of their neighbors, effectively lightening the can and reducing its “moment of inertia.” But those atoms would have to stand still as the rest of the helium moved, so they’d have to flow through the solid unimpeded.

In 2006, however, Reppy and a colleague showed that they could reduce the size of the frequency shift if they warmed their helium to close to its melting point (*Science*, 24 March 2006, p. 1693). Such warming, called annealing, repairs defects in the pattern of atoms in a crystalline solid. So if atoms were flowing, it had to be along defects in the crystal, physicists concluded.

But Reppy’s new result suggests that nothing flows. This time, instead of annealing his

Review Letters. Rather, it reduced the frequency at higher temperatures. “And that’s completely contrary to the supersolid picture,” he says.

It’s possible to explain the observed frequency shifts without flow, Reppy says. Adding defects may make the solid helium softer so that it acts less like a chunk of diamond and more like a blob of Jell-O that sloshes as the oscillator twists back and forth. That sloshing would reduce the frequency of twisting. The effect would go away at low temperatures as the defects lock up and the solid stiffens.

How did physicists mistake a frequency decrease at higher temperatures for a frequency increase at lower temperatures? Previous experiments relied on annealing to change the density of defects. But the process causes the helium to settle and generally creates a large, uncontrolled frequency shift that researchers must subtract before comparing data taken before and after annealing, Beamish says. Thinking that all the action was at low temperatures, physicists had assumed that plots of frequency versus temperature should coincide at high temperatures (see figure).

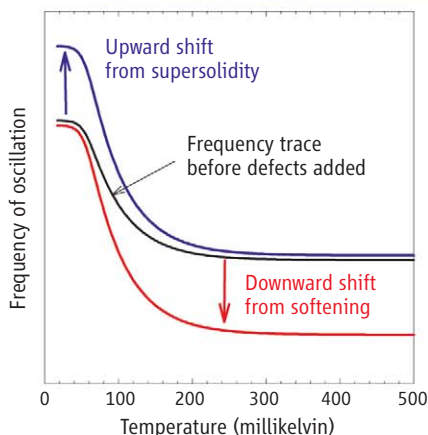
So is this the end of supersolid helium? Not necessarily, Chan says: “I don’t see anything wrong with John’s measurement, but probably it can’t be taken as evidence that the whole idea of supersolidity is gone.” For example, he says, softening does not explain signs of flow seen in helium crammed into the nanometer-sized pores of a type of glass, where the stiffness of the helium shouldn’t make a difference.

Moreover, Chan notes, theorists think that the flow in supersolidity may occur along stringlike defects called dislocations, but only after they have frozen into place. So higher-temperature softening could be related to lower-temperature flow. Reppy’s cell squeezes the helium into a very thin annulus, which may accentuate the effects of softening over those of flow, Chan says.

The new experiment may only replace one puzzle with another. To fully explain the frequency shifts Reppy sees with softening, the helium would have to become almost as squishy as a liquid, Beamish says. And it’s not clear why solid helium should be so soft. “It’s completely miraculous,” Reppy says, “as miraculous as the supersolid effect in terms of trying to understand it.” Stay tuned; the mystery of solid helium continues to deepen.

—ADRIAN CHO

TWO INTERPRETATIONS



Which way? If solid helium flows, then adding defects to it should make this torsional oscillator twist faster at low temperatures (blue trace). Instead, it twists slower at higher temperatures (red trace).

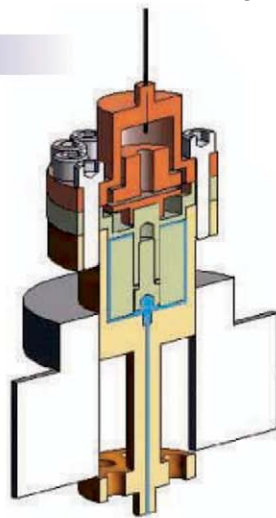
and the proposed softening may arise from the same underlying mechanism and that, by virtue of its design, Reppy’s experiment may accentuate the latter.

Liquid helium—specifically the isotope helium-4—flows without resistance when chilled below 2.17 kelvin as the light atoms in it collect in a quantum wave. Since the late 1960s, some theorists had speculated that might still happen even when the liquid is pressurized to 25 times atmospheric pressure to form a solid.

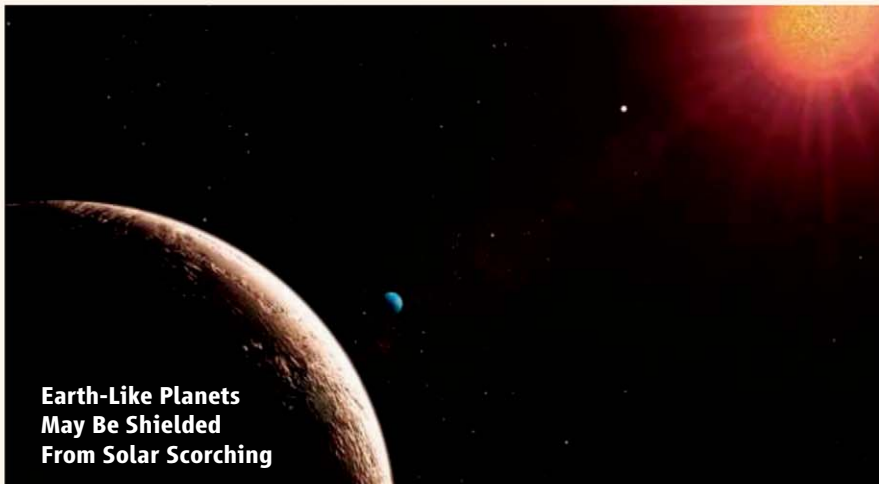
In 2004, Moses Chan of Pennsylvania State University, University Park, and a colleague reported signs of such flow in solid helium.

helium to reduce defects, he pushes on it to create more. The 79-year-old don of low-temperature physics created a torsional oscillator that holds an annulus of solid helium between an outer cylinder and an inner one that moves like a piston. By moving it by a micrometer, Reppy could deform and add defects to a sample he’d already studied.

If atoms really do flow freely along defects, then adding more defects should allow more flow, and at low temperatures the frequency of twisting should soar even higher. But adding defects did not increase the frequency at low temperatures, as Reppy reported online on 21 June in *Physical*



ScienceNOW

From *Science's* Online Daily News Site

Earth-Like Planets May Be Shielded From Solar Scorching

Many of our galaxy's suns have destroyed the atmospheres of orbiting Earth-like planets—or so astrobiologists have long feared. The Milky Way, they note, is dominated by M dwarf stars: violent, unpredictable suns that frequently hurl high-energy particles and solar flares into space. Because they are much cooler than our sun, any potentially habitable planet would need to orbit them much closer than Earth does, putting it smack in the danger zone. But a new study indicates that these planets may be unexpectedly shielded from solar activity, keeping life safe. <http://bit.ly/other-earths>

Lightest Bits of Matter Just Got Lighter

Cosmologists have used measurements of some of the most massive objects in the universe to place a limit on the mass of the lightest particle in the cosmos. Using data from a survey of 700,000 galaxies, the researchers found that an elusive subatomic particle called a neutrino can have a mass of no more than 0.28 electron volts, which is less than one-billionth of the mass of a hydrogen atom. <http://bit.ly/lighter-mass>

Filoviruses Go Way Back

Relatives of the lethal Ebola and Marburg viruses likely began infecting mammals tens of millions of years ago, according to a new study, making this family of viruses, known as filoviruses, far older than scientists had thought. By comparing viral remnants in different species, scientists found they were nearly identical, indicating that they infected mammals only once early in evolution, and then the viral remnants were passed down as the groups diverged. Remnants of genes from these viruses exist in the DNA of bats, marsupials, rodents, and other mammals, a finding that may suggest where these deadly microbes lurk before they emerge to kill people. <http://bit.ly/filovirus>

Tanning Ability Driven by Evolution

Sunbathers who bronze beautifully have natural selection to thank. Research published in the *Proceedings of the National Academy of Sciences* suggests that the ability to tan is a trait that evolved several times in mid-latitude regions, such as China and the Mediterranean, where the sun's intensity varies dramatically from season to season. If inhabitants of these regions had consistently dark skin, which blocks the sun's rays, they wouldn't have produced enough vitamin D in the winter. If they had consistently light skin, their bodies would have been robbed of folate, a light-sensitive vitamin essential for cell division and repair. Folate is especially important during pregnancy; too little can result in birth defects. Indeed, the researchers posit that sun-induced folate deficiency, rather than skin cancer or sunburn, was the driving force behind the evolution of dark skin and tanning. <http://bit.ly/tan-evolve>



Read the full postings, comments, and more at news.sciencemag.org/sciencenow.

ScienceInsider

From the *Science*
Policy Blog

The Supreme Court this week struck down a controversial business patent in its ruling on *Bilski v. Kappos*. But advocates who wanted the court to spell out clear rules on the **patentability of inventions that deal with methods and processes** were disappointed with the ambiguous ruling. Biotech advocates, fearing new barriers on intellectual property, were pleased. <http://bit.ly/bilsky-ruling>

President Barack Obama this week released a **National Space Policy** that affirms the Administration's commitment to commercial space flight and exploration. The policy gives the mid-2030s as a target for the first human flight to Mars and promises a human mission to a nearby asteroid in 2025. http://bit.ly/space_policy

Marine scientist Sean Powers says he's seeing more oil and less of a **key seaweed called *Sargassum*** in the wake of the gulf oil spill. Meanwhile, the federal government halted a Louisiana plan to build giant sand berms to protect wetlands. <http://bit.ly/sargassumoil> and <http://bit.ly/islandsdredge>

Former University of Wisconsin, Madison, biologist Elizabeth Goodwin pleaded guilty to **falsifying data** on a grant progress report. She will pay \$50,000 and be barred from federal research for 3 years. A group of her graduate students reported the misconduct. <http://bit.ly/Goodwinplea>

Kentucky agrarian literary legend **Wendell Berry** has **pulled his personal papers** from the University of Kentucky in protest over the school's emphasis on research over teaching and its environmental policies. "The University of Kentucky has a mandate to look after the country people and the rural landscapes," he told *Insider*. <http://bit.ly/berrykentucky>

Presidential science adviser John Holdren says that a **report on scientific integrity**, nearly a year late, will simply augment efforts already in place that are aimed at improving government performance on the issue. President Obama had requested a full "plan" to tackle the problem. <http://bit.ly/integritytardy>

For the full postings and more, visit <http://news.sciencemag.org/scienceinsider>.

GULF OIL DISASTER

Hunting for Plumes, Learning to Live in a Media Spotlight

Not long after the *Deepwater Horizon* rig burned and sank, Samantha Joye had a hunch about how the impending disaster would unfold. As oil and gas gushed out of the ruptured well into the Gulf of Mexico, she reasoned, many of the hydrocarbons would spread underwater in deep plumes rather than all rise to the surface, as many expected. But she had no inkling of what her confirmation of this insidious subsurface dispersal would mean for her life and career in the crazy weeks and months to follow.

Thanks to a combination of expert insight and the good fortune of having the first research vessel out of the docks, Joye and her colleagues were the earliest of several groups to find evidence of the plumes. For Joye, a biogeochemist at the University of Georgia, Athens, the impact of the discovery was immediate and unrelenting. Since the findings made national headlines in mid-May—after Joye tipped off a reporter from *The New York Times*—she has been the focus of persistent media attention. “This dwarfs anything I could have imagined,” says Joye, who has become one of the chief independent scientific voices of the spill. “I think she’s done a great service drawing attention to issues that otherwise would have possibly gotten swept under the rug,” says chemist Jeffrey Short of Oceana, an advocacy group in Washington, D.C.

Joye, 44, first heard rumors that something was badly amiss with an oil platform from colleagues who had spotted large columns of smoke while doing research nearby. Shortly after the oil rig sank, Joye e-mailed the program manager who was handling her upcoming cruise on the *R/V Pelican*, which was being funded by the National Oceanic and Atmospheric Administration (NOAA). The original plan had been to map gas vents and other features on the sea floor, but Joye wanted to change the focus to the impact of the oil spill. He quickly agreed.

Based on her previous studies of naturally released hydrocarbons in the gulf, Joye suspected that oil and gas from the leaking well would become entrained in the water column and then move laterally with the prevailing currents. A bad back prevented Joye from going on the cruise, but she helped coordinate research and real-time analyses from her office.



Reluctant star. Samantha Joye has become one of the most-quoted scientists on the oil spill.

On 12 May, scientists on the *Pelican* detected signals that indicated an unusual layer of dissolved organic matter that suggested plumes between 700 and 1300 meters deep. Oxygen levels were lower than normal in the suspected plumes, probably because bacteria were metabolizing methane gas. Back in Georgia a few days later, Joye called the *Times* reporter, who wrote about the finding on 15 May. The story described Joye’s alarm: “There’s a shocking amount of oil in the deep water, relative to what you see in the surface water,” she said.

Reporters eagerly chased the front-page story. Just 10 minutes after the story went online, Joye’s phone began to ring. Reporters kept calling until 2 a.m., she says, when she unplugged the phone. News trucks were parked in front of her office on the campus. “I didn’t have a minute’s peace,” she says. “It was absolutely insane.”

The media maelstrom did not go unnoticed at NOAA. On Monday, 17 May, NOAA Administrator Jane Lubchenco released a cautious statement emphasizing that the *Pelican* cruise hadn’t yet confirmed the presence of oil in the plumes and that the falling oxygen levels weren’t an immediate danger to marine life. Lubchenco added: “We eagerly await results from their analyses and share

with them the goal of disseminating accurate information.” In a subsequent lecture at a large research meeting, while discussing preliminary plume measurements from a NOAA vessel, Lubchenco warned the audience: “If we jump to conclusions, that doesn’t serve science well. Verification, not conjecture, is what I would urge.”

Joye and others were puzzled by the reaction to the *Pelican* findings. “A lot of academicians were surprised by NOAA’s behavior,” says Ernst Peebles of the University of South Florida in St. Petersburg,

who has also identified plumes in the gulf. Ian MacDonald of Florida State University, Tallahassee, felt that NOAA was “basically challenging Samantha’s interpretation of this data.” NOAA says it wanted to correct misleading news stories and put out accurate information. “The intent was to distinguish what we actually knew from the cruise from speculation,” according to Steve Murawski of NOAA, who heads an interagency group on subsurface sampling.

Peebles thinks Joye has prompted NOAA to be more open; the agency now regularly puts out reports on preliminary results from cruises. “NOAA has changed its policy ... as a result of interaction with the *Pelican* cruise, for the better,” he says. NOAA’s most recent report, released last week, confirms the presence of plumes at a concentration of 1 to 2 parts per million within a few kilometers of the well.

Joye says she was also motivated to keep talking with reporters because she was frustrated that more research wasn’t under way on tracking the hydrocarbons and their impact in the deep sea. Since then, she has been using her newfound soapbox to advocate for more research, even testifying on Capitol Hill.

Just a few days after the NOAA statement, Joye got an unexpected chance to do more science. The University of Miami’s *R/V F. G. Walton Smith* had become available for a research cruise, but she had only 4 days to organize the mission—a task that normally takes more than 2 months. “It wasn’t the most pleasant cruise, but I had to be out there.” To manage her back pain, she brought along a bottle of ibuprofen—the extra-large pills she normally gives to her horse.

The team of 11 scientists from four institutions worked around the clock for 2 weeks, collecting samples to track and analyze the

plume. As Joye described in her daily blog* from the cruise and in press conferences, the team found the first visible signs of oil in the deep-water samples, found methane concentrations up to 100,000 times higher than normal, and discovered that oxygen levels were as much as 40% below normal. Press interest

*<http://gulfblog.uga.edu/>

remained high: A TV news crew chartered a boat to visit the ship at sea; more were waiting at the docks when the ship returned.

Joye's main goals right now are to figure out whether the microbes are feeding more on oil or gas and which nutrients might limit their action; these factors likely determine how much oxygen the microbes are using, which is important to understand possible

impacts on other marine life. She's hoping to get another cruise next month and ideally take a submarine into the plume in August to look for impacts on fish and other marine life. "It's a disaster, and I love the Gulf of Mexico," she says. "I'm going to do whatever I have to do to figure out what's going on out there."

—ERIK STOKSTAD

With reporting by Lauren Schenkman.

GULF OIL DISASTER

How to Kill a Well So That It's Really Most Sincerely Dead

After a run of "top kill" attempts failed to stanch the flow of crude oil from its Macondo well in the Gulf of Mexico, oil giant BP has one major option left: plug the flow near its source by drilling into the bottom of the runaway well. That will require hitting an 18-centimeter-wide well 5500 meters beneath the gulf's surface. It may sound like a tall order, but drillers are confident. It "is going to be the ultimate answer," says Paul Bommer, a petroleum engineer at the University of Texas (UT), Austin. The technology "is miraculous," adds Bommer's UT Austin colleague Tadeus Patzek. "It's difficult, it's going to take time, and it will be frustrating. [But] eventually they'll get there, absolutely." And a kill looks almost as certain.

A bottom kill may take months of drilling to reach its target, but it has an innate advantage over the top-down approach.

As in a top kill, the goal is to fill the well with enough heavy drilling "mud" to counter the pressure of oil and gas rushing up from the reservoir below. Mud injected at the top of the well needs enough extra back pressure to slow the flow so it can sink down the well. That never happened on BP's repeated attempts. In a bottom kill, by contrast, the rising oil and gas can actually give the mud a boost.

First, however, the drillers must find the bottom of the target well. They start with a navigation device invented for submarines and missiles, says borehole geophysicist Roger N. Anderson of Columbia University's Lamont-Doherty Earth Observatory in Palisades, New York. Twenty years ago, workers at Los Alamos

National Laboratory developed a set of accelerometers so compact they could be installed in a missile, or just above a drill bit. By precisely measuring acceleration in three dimensions throughout drilling, the navigation package can tell drillers where the drill bit is within several meters at all times, says Anderson.

Another technology, directional drilling, lets drillers act on that precise navigation information. The fastest method for turning a borehole in a preferred direction is the "point the bit" approach, says Bommer. The entire string of drill pipe is turned from the drilling platform; in addition, the assembly at the bottom of the drill string can be set to give the bit an "extra bump" in the preferred direction with each rotation. Knowing the location of both drill and target within a few meters isn't good enough when drilling into

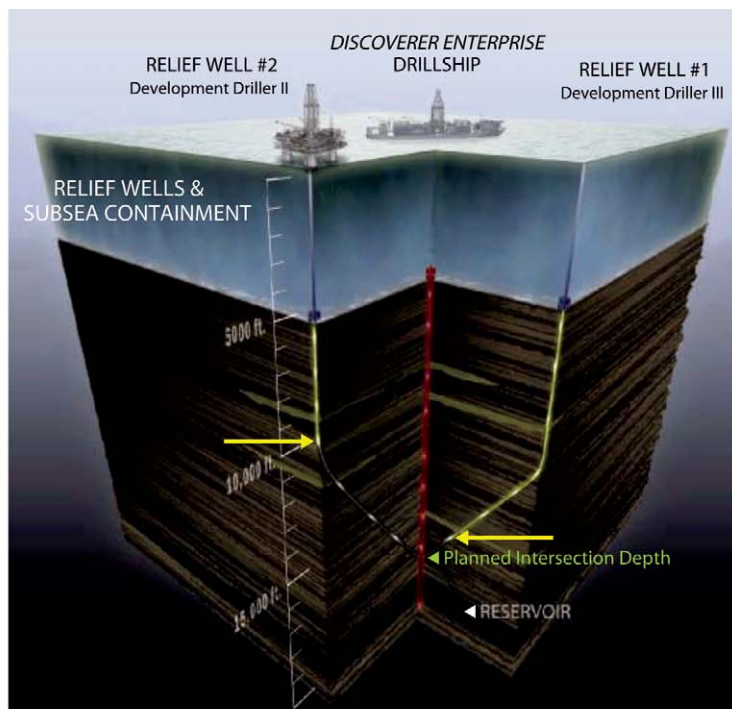
an 18-centimeter wellbore, so drillers eventually bring into play an active technique for locating the target well.

In the current operation, BP's drillers started their relief well (the first of two the federal government required) 850 meters from the Macondo well. They drilled about 3660 meters straight down and then bent toward the target well. Using an electromagnetic technique that senses the steel casing of the well, they steered the drill bit to 6 meters from the Macondo well and are now drilling parallel to it with another 300 meters to go to the intended intersection point. Ranging continuously, drillers will home in on the intersection point in the last 60 meters of drilling.

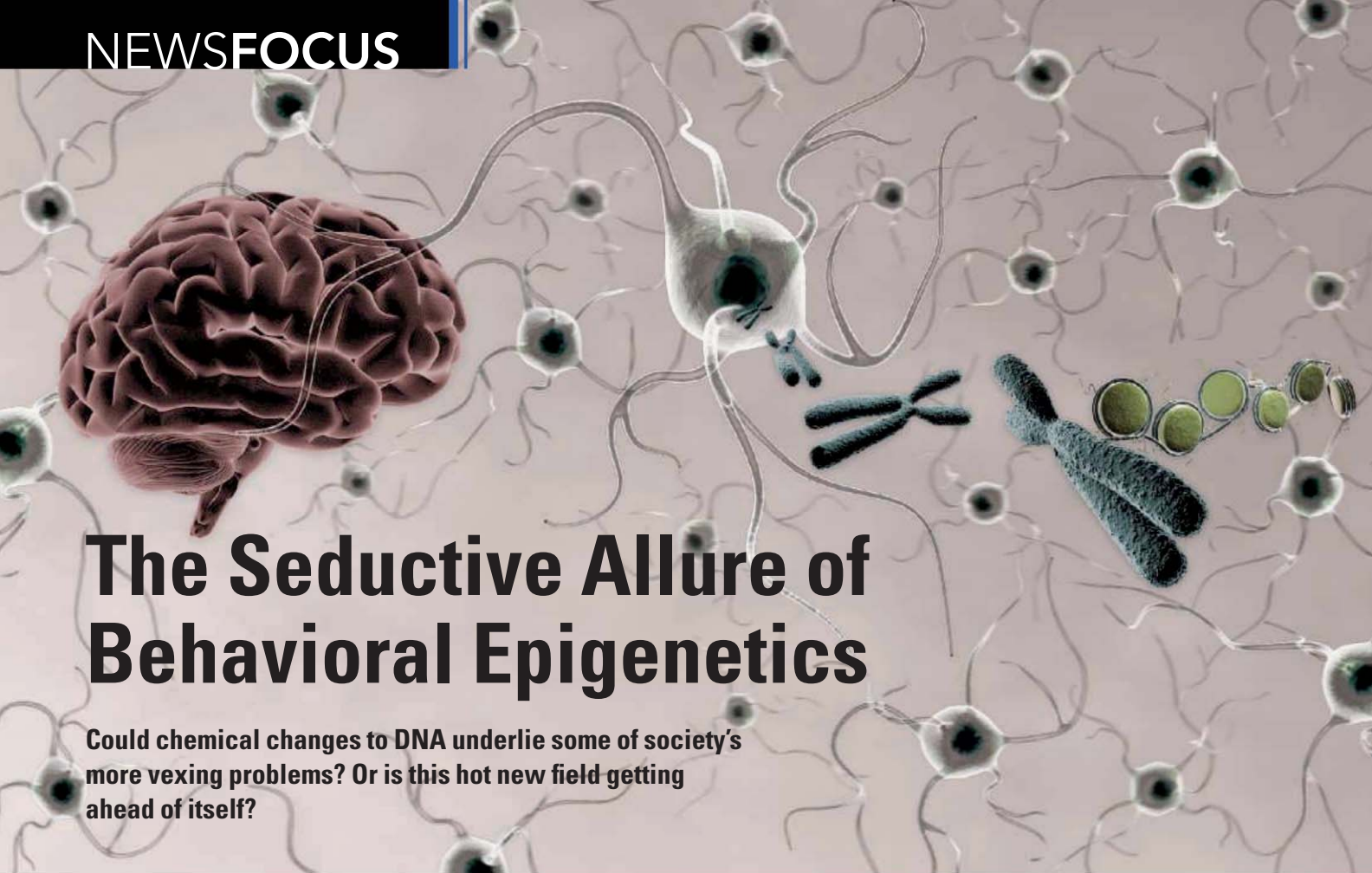
"This is the point when we have to be very good, and we are," BP Senior Vice President Kent Wells said at a press briefing on 28 June. The record bears him out. As Wells noted, John Wright of Boots & Coots International Well Control Inc., which is running the relief well operation, has overseen 40 relief wells and borehole intersection operations. All 40 successfully intersected their targets. "It's not so much 'if' but 'when' the well is intersected, said Wells.

Intersection is not a kill, however. Patzek cautions that the way this well appears to have failed—at the point where cement was supposed to have sealed the space between two steel pipes—may make stanching the flow more difficult than usual. With such potential complications in mind, Anderson puts the odds of a kill using two wells at 98%. There are no blowouts gushing endlessly, Anderson notes. "They will succeed," he says. "The only question is how long it will take."

—RICHARD A. KERR



On target. Two relief wells (yellow) are being guided toward the gushing Macondo well (red) by drillers using onboard navigation and remote-sensing instrumentation.



The Seductive Allure of Behavioral Epigenetics

Could chemical changes to DNA underlie some of society's more vexing problems? Or is this hot new field getting ahead of itself?

MICHAEL MEANEY AND MOSHE SZYF WORK in the same Canadian city, but it took a chance meeting at a Spanish pub more than 15 years ago to jump-start a collaboration that helped create a new discipline. Meaney, a neuroscientist at the Douglas Mental Health University Institute in Montreal, studies how early life experiences shape behavior later in life. Across town at McGill University, Szyf is a leading expert on chemical alterations to DNA that affect gene activity. Sometime in the mid-1990s, both men attended the same meeting in Madrid and ended up at a bar talking and drinking beer. "A lot of it," Szyf recalls.

Meaney told Szyf about his findings that rat pups raised by inattentive mothers tend to be more anxious as adults than pups raised by more nurturing mothers. He also described how the activity of stress-related genes was altered in the undernurtured pups. At some point in the conversation, Szyf had a flash of insight: This difference must be due to DNA methylation—the chemical alteration he had been studying in stem cells and tumor cells.

The idea cut against the conventional thinking in both fields. In neuroscience, the prevailing wisdom held that long-term changes in behavior result from physical changes in neural circuits—such as when neurons build new synapses and become more sensitive to

messages from their neighbors. And most scientists who studied DNA methylation thought the process was restricted to embryonic development or cancer cells.

Back in Montreal, the pair eventually began collaborating, and Szyf's hunch turned out to be right. Their research, along with work from a handful of other labs, has sparked an explosion of interest in so-called epigenetic mechanisms of gene regulation in the brain. Long familiar to developmental and cancer biologists, these molecular mechanisms alter the activity of genes without changing their DNA sequence (*Science*, 10 August 2001, p. 1064). And they have recently become a white-hot topic in neuroscience. Meaney and Szyf's work suggests that epigenetics could explain how early life experiences can leave an indelible mark on the brain and influence both behavior and physical health later in life. These effects may even carry over to subsequent generations. Meanwhile, other researchers have implicated epigenetics in drug addiction. Still others have described important roles in cognition (see sidebar, p. 27).

Some researchers speculate that if these rodent findings extend to humans, epigenetics could turn out to be at the heart of some

of the most vexing problems in society. These ills include the long-term health problems of people raised in lower socioeconomic environments, the vicious cycle in which abused children grow up to be abusive parents, and the struggles of drug addicts trying to kick the habit.

Tempting as such speculation may be, others worry that the young but fast-growing field of behavioral epigenetics is getting ahead of itself. They point out that so far there's very little evidence in humans that epigenetics connects early life experience to behavioral or health problems later in life. Moreover, several experimental obstacles will make finding proof exceedingly difficult. "I think there's been a lot of putting the cart before the horse," says Gregory Miller (no relation), a psychologist at the University of British Columbia (UBC), Vancouver.

The importance of a loving mother

In 2004, Szyf and Meaney published a paper in *Nature Neuroscience* that helped launch the behavioral epigenetics revolution. It remains one of the most cited papers that journal has ever published. The paper built on more than a decade of research in Meaney's

Online
sciencemag.org
Podcast interview
with author
Greg Miller.

CREDITS: (BRAIN) ISTOCKPHOTO; (ILLUSTRATION) Y. GELMAN/SCIENCE; (COLLAGE) N. KEVITYAGALA/SCIENCE

lab on rodent mothering styles.

Rat moms vary naturally in their nurturing tendencies. Some lick and groom their pups extensively and arch their backs to make it easier for their young to nurse. Others spend far less time doting on their pups in this way.

Meaney had found that the type of mothering a rat receives as a pup calibrates how its brain responds to stress throughout its life. Rats raised by less-nurturing mothers are more sensitive to stress when they grow up. When confined to a Plexiglas tube that restricts their movement, for example, they exhibit a greater surge in corticosterone, a hormone pumped out by the adrenal glands in times of stress. The likely cause is reduced numbers of a receptor for steroid hormones in the brain. This so-called glucocorticoid receptor is part of a negative feedback loop that dials down the volume on communication between the brain and adrenal glands, thereby reducing reactivity to stress.

The *Nature Neuroscience* paper linked this reduction in glucocorticoid receptors to DNA methylation. Rats raised by less-nurturing moms tended to have more methyl groups attached to the promoter region, the “on” switch, of the glucocorticoid receptor gene. These methyl groups block access by the transcription factors that turn the gene on. As a result, fewer receptors are produced. Subsequent experiments showed that enzymes that reverse DNA methylation of the glucocorticoid receptor gene also reverse the effects of unenthusiastic mothering on the offspring’s hormonal and behavioral responses to stress.

Several of Meaney’s students have carried on with this work and extended it in new directions. Frances Champagne, a co-author of the 2004 paper, went on to show that female rats raised by nurturing mothers are more nurturing mothers themselves. She also found that pups raised by less-nurturing moms exhibit greater methylation—and reduced expression—of the gene for a particular estrogen receptor in the hypothalamus, a brain region involved with reproductive behavior. This receptor amplifies signaling by oxytocin, a hormone that promotes mother-infant bonding.

Now at Columbia University, Champagne has been investigating the long-term effects of other kinds of social experiences early in life. She has found that mice raised communally by multiple mothers (as rodents raise their young in the wild) are better socially adjusted as adults: They are less likely to pick a fight with a stranger put into their cage, for example. Communally raised mice also spend

more time fussing over their own offspring. Fussled-over daughters in turn tend to grow up to be nurturing mothers. These changes correlate with a higher density of oxytocin receptors in some brain regions, the researchers reported last September in *Frontiers in Behavioral Neuroscience*. Champagne’s lab is now investigating whether that higher density could be due to epigenetic modifications. “What’s exciting to me is that the social world, which can be perceived as being this ethereal thing that may not have a biological basis, can affect these mechanisms,” she says.

Adversity takes its toll

Whereas a nurturing environment can predispose a rodent to be calmer in adulthood and raise a nurturing family of its own, an adverse environment can have the opposite effect. There’s evidence that this effect, too, may involve epigenetic changes. Last year, researchers led by Tania Roth and J. David Sweatt of the University of Alabama, Birmingham, helped show this by building on earlier work showing that rat mothers denied access to the materials needed to make a proper nest become anxious and spend less time nurturing their young. Pups raised by these stressed-out rat moms exhibited increased methylation of the gene for BDNF, a neural growth factor, in the brain’s prefrontal cortex, they reported in the 1 May 2009

issue of *Biological Psychiatry*. In addition, this methylation pattern, which would tend to reduce the amount of BDNF produced, was passed on to the subsequent generation.

Exactly what low BDNF levels mean for a mouse isn’t entirely known. However, levels of this growth factor are reduced in mouse models of depression and anxiety, at least in certain brain regions, and restoring it can mimic the effect of antidepressant drugs. In 2006, Eric Nestler, currently at Mount Sinai Medical Center in New York City, and colleagues reported in *Nature Neuroscience* that the *Bdnf* gene is down-regulated in the hippocampus of adult mice exposed to social stress—in the form of chronic bullying by a bigger mouse. In the same paper, Nestler’s team linked this reduction in *Bdnf* activity to epigenetic modifications involving histones, tiny protein spools that keep DNA wrapped up. Chronic stress triggered an increase in a type of histone methylation that suppresses gene activity by keeping the DNA containing the *Bdnf* gene tightly wound. Antidepressant drugs, on the other hand, boosted histone acetylation, which helps unwind DNA from histones and promote *Bdnf* activity. Such findings hint that epigenetic modifications could be an important link between adverse life experiences and the risk of psychiatric disorders such as depression and anxiety, Nestler says.



Different upbringings. Being raised by a nurturing (top left) or a lackadaisical (top right) mother can cause epigenetic differences that affect a rat pup’s behavior later in life. Whether similar differences occur in people raised in wealthy (bottom left) or impoverished (bottom right) neighborhoods remains an open question.

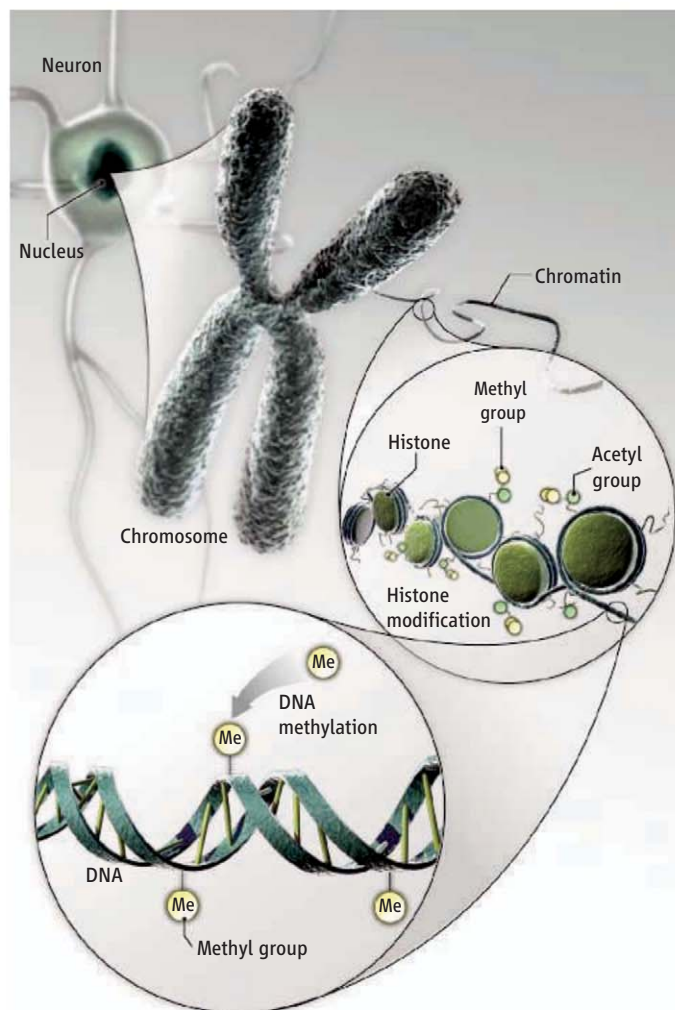
Another line of work in Nestler's lab suggests that epigenetic mechanisms could also play an important role in drug addiction. His team has now documented several epigenetic changes provoked by cocaine administration in rodents. The drug increases acetylation and decreases methylation of histones—both of which tend to promote gene activity—in the brain's reward circuitry. "Cocaine produces changes in the genome that make the brain more sensitive to the next dose of cocaine," Nestler says.

For example, in the 8 January issue of *Science* (p. 213), Nestler and colleagues reported that repeated cocaine administration suppresses methylation of a particular histone in one reward region, the nucleus accumbens. Suppressing this type of histone methylation in mice that had never received cocaine triggered the growth of extra dendritic spines—tiny extensions on neurons that tend to sensitize them—in the nucleus accumbens. Suppressing methylation also increased the animals' preference for cocaine once they finally tried it. In contrast, enhancing histone methylation reduced cocaine-seeking behavior. Nestler cautions that any treatments for human drug addicts are a long way off, but he says a better understanding of the genes altered by such epigenetic changes could eventually point to new treatments to break the hold of addiction.

The human story

If the rodent research on epigenetics translates to humans, the implications could be far-reaching. The effects of adverse environments early in life are well documented and notoriously hard to shake. Childhood abuse, for example, elevates the lifelong risk of depression, anxiety, and suicide. Growing up in an impoverished environment also takes a lasting toll, affecting physical health as well as behavior. Could epigenetics be part of the reason?

So far, the evidence in humans is scant. A major reason, and a huge obstacle to the field in general, is that human brain tissue is hard to come by. In one of the few studies to date, Meaney, Szyf, and postdoctoral fellow Patrick McGowan examined post-



Epigenetic breakdown. Several epigenetic mechanisms alter gene activity in neurons, with potentially important effects on brain function and behavior. Histone acetylation tends to promote gene activity, whereas histone methylation and DNA methylation tend to inhibit it.

mortem brains from 24 people who had committed suicide, half of whom had been abused as children. From their rodent work, the researchers hypothesized that those who had been abused might have more methyl groups on the glucocorticoid receptor gene than did those who had not been abused. That's indeed what they found, the researchers reported in the March 2009 issue of *Nature Neuroscience*.

Lacking access to brain tissue, researchers have looked elsewhere in the body for signs of epigenetic alterations. In the March-April 2008 issue of *Epigenetics*, a team led by Tim Oberlander at UBC reported increased methylation of the glucocorticoid receptor gene in cells isolated from umbilical cord blood in 33 infants born to women who suffered symptoms of depression during their pregnancy. Those infants also had higher cortisol concen-

trations in their saliva when tested 3 months later, suggesting an increased susceptibility to stress.

"Those data are very consistent with the rodent work, although there's some ambiguity about what's actually going on," says UBC's Miller, who was not involved with the study. One issue, he and others point out, is that cord blood contains a mishmash of different cell types. Methylation patterns can vary significantly from one cell type to another, and the proportion of cell types in cord blood can vary significantly from one individual to another. As a result, Miller says, the findings in the *Epigenetics* paper could be accounted for by a difference in the combination of cell types sampled rather than a difference in methylation in any particular cell type. "That's a real problem for that study and others like it," he says.

In Vancouver, Miller and UBC colleagues Edith Chen and Michael Kobor head an ongoing study of gene expression in people raised in different socioeconomic conditions. Their work focuses primarily on white blood cells. Miller says he expected to find increased methylation of the glucocorticoid receptor gene studied by Meaney's group, reasoning that the lower socioeconomic environment of his

subjects might be roughly analogous to the un-nurturing environment of Meaney's rat pups. So far they haven't found it. "We're not seeing anything in the way of DNA methylation in the glucocorticoid receptor [gene]," he says. One possible explanation, Miller says, is that blood and brain cells don't necessarily undergo the same epigenetic changes in response to a given life experience.

Miller also suspected that he and his colleagues would find epigenetic alterations in genes related to physical health. Because of its long-lasting effects on gene activity, DNA methylation seemed like a potentially attractive explanation for why people who grow up in poor households have an increased lifetime risk of health problems. (A 2006 study of graduates of Johns Hopkins University School of Medicine, for example, showed that even in this well-educated, affluent population, those who'd grown up in poor house-

holds decades earlier had 2.4 times the risk of heart disease compared to those who'd grown up in wealthier households.)

Socioeconomic status early in life does appear to alter gene expression: In a 25 August 2009 paper in the *Proceedings of the National Academy of Sciences (PNAS)*, Miller and colleagues reported disparities in the activity of more than 100 genes related to immune system function in the white blood cells of men who lived in lower socioeconomic environments before the age of 5. The net result of these changes in gene activity would tend to increase inflammatory immune responses, a potential contributing factor to the documented increases for infectious and cardiovascular diseases related to poverty.

But epigenetics does not seem to be the cause of these changes. "We spent the last few years trying to see if we could find evidence of epigenetic alterations in the immune system that are related to early life experience," Miller says. "This work is still ongoing, so I think it would be premature to conclude any-

thing definitively, but we've had less success than we'd hoped and imagined."

Others also feel that too much emphasis is being put on epigenetics as the link between environment and genes. "The big-picture story is that clearly social interactions can regulate gene expression, but they do so in different ways in different tissues," says Steve Cole, a genomics researcher at the University of California (UC), Los Angeles, who collaborated on the *PNAS* study. Cole notes that epigenetics represents only one class of potential mechanisms for altering gene activity. He argues that changes in the activity of transcription factors can cause long-term changes in gene expression without help from DNA methylation or other epigenetic mechanisms.

The excitement over the Meaney findings has led many researchers to look for epigenetic alterations, but Cole says he knows of several others who are coming up empty-handed: "Lots of people have spent lots of time and money and are now a little grumpy about this."

At UC Berkeley, one of Meaney's former students, Darlene Francis, says she has mixed feelings too. "These phenomena are really exciting from a public-health perspective," says Francis, a neuroscientist and public-health researcher. Epigenetics provides a potential explanation for how social conditions can affect biology in ways that can contribute to poor health, Francis says: "This allows folks who are convinced that social forces are huge contributors to risk and vulnerability to make more effective arguments." At the same time, Francis says too many researchers are embarking on "undirected" searches for epigenetic alterations in human populations without a solid rationale. "What some people take away [from the rodent work] is that methylation is now the cause and solution to a lot of life's problems," she says. "I get frustrated with the overextrapolation of the animal findings, and some of it is my work so it's ironic," Francis says. "I don't know when I became the sensible one."

—GREG MILLER

A Role for Epigenetics in Cognition

The push to show that epigenetics can translate early life experiences into lasting changes in behavior (see main text, p. 24) has been accompanied by a parallel surge of interest in how chemical modifications to DNA can affect cognition. This work sprang from research in the late 1990s showing that abnormalities in DNA methylation are involved in developmental disorders that cause intellectual impairment, including Angelman syndrome and Rett syndrome, says J. David Sweatt, a neurobiologist at the University of Alabama, Birmingham. Sweatt's lab and others have since found evidence that epigenetic mechanisms play important roles in learning and memory in adult rodents. One recent study even suggests that these mechanisms may help explain why memory declines with age.

Sweatt's team recently discovered that training mice to associate a certain location with a mild electric shock reduced DNA methylation in the hippocampus, a brain region crucial for memory formation. More specifically, the *Bdnf* gene was demethylated, boosting its activity, they reported 15 October 2008 in *The Journal of Neuroscience*. This gene encodes a growth factor that promotes new synaptic connections between neurons so that a memory is retained. Injecting a drug that inhibits demethylation of DNA into the hippocampus prevented the increase in *Bdnf* activity after learning and weakened rodents' memory of the place where they had received a shock. The work is one of several clues that DNA methylation affects the formation and maintenance of memories.

Other work has focused on histone acetylation, a chemical modification that unwinds DNA from protein spools called histones, thereby enabling gene activity. One of the most intriguing studies to date was led by Li-Huei Tsai and André Fischer at the Massachusetts Institute of Technology in Cambridge. In the 10 May 2007 issue of *Nature*, they reported that a drug that promotes histone acetylation improved learning and memory in a mouse model of Alzheimer's disease. When injected into the hippocampus, the drug even seemed to restore a forgotten memory of a location where the rodents had previously received a shock.



Maintaining memories. Epigenetic mechanisms in the brain seem to play important roles in memory and may be an attractive target for drugs to stave off memory loss in old age.

More recently, Fischer, who is now at the European Neuroscience Institute in Göttingen, Germany, has been investigating alterations in histone acetylation that occur naturally with age. In the 7 May issue of *Science* (p. 753), he and colleagues reported that adult mice, compared with juveniles, exhibit reduced histone acetylation and diminished activation of genes in the hippocampus that were related to learning and memory. As in the Alzheimer's mice, drugs that boosted histone acetylation improved the older mice's performance on tests of rodent cognition.

Biotech and pharmaceutical companies are already exploring these drugs, called histone deacetylase inhibitors, for treating Alzheimer's disease, says Ottavio Arancio, a neuroscientist at Columbia University. Some of these drugs are already approved for treating cancer. However, because the drugs alter the activity of multiple genes, Arancio cautions that more work is needed to determine whether they can aid memory without causing serious side effects.

—G.M.



PROFILE: DOLORES PIPERNO

In Archaeobotanist's Hands, Tiny Fossils Yield Big Answers

Dolores Piperno's microscopic techniques overcame skeptics and revolutionized views of early agriculture in the Americas

WASHINGTON, D.C.—Some scientific breakthroughs are the result of lucky breaks. And then there are those that come when researchers know exactly what they're looking for and where and how to find it.

That's what happened last year, when archaeobotanist Dolores Piperno and her co-workers reported in the *Proceedings of the National Academy of Sciences* finding the earliest known maize (corn) deep in the Balsas River Valley of southern Mexico. Geneticists had traced the origins of maize to these lowlands and estimated that humans domesticated it about 9000 years ago. Yet their findings seemed to clash with the archaeological evidence: Radiocarbon dates put the earliest maize cobs, from the Mexican highlands, at only about 6200 years ago.

But Piperno and her team weren't looking for cobs, which disintegrate in the tropical humidity. Instead, armed with techniques that Piperno had perfected over many years, they searched for stone tools to which traces of maize might have adhered. In a Balsas Valley rock shelter, they found just that. A trove of grinding stones, closely associated with charcoal radiocarbon dated to 8700 years ago, harbored microscopic fossils of maize. For many researchers, the findings reconciled the genetic and archaeological evidence and ended a long debate

about whether maize had been domesticated in the highlands or the lowlands.

"That's exactly how you're supposed to do science," says archaeobotanist Deborah Pearsall of the University of Missouri, Columbia. "If you look at the corpus of Dolores's work, you see the power of a scientist who chooses her research topics on the basis of hypotheses she wants to test."

Along the way, Piperno, who has a joint appointment at the Smithsonian Institution's National Museum of Natural History here and its Tropical Research Institute (STRI) in Balboa, Panama, has persuaded many former skeptics to accept early dates for farming in the Americas. One such is archaeobiologist Bruce Smith, also of the Smithsonian. "I did have concerns regarding the dating" and identification of plant microfossils, Smith says. "But [microfossil] approaches have revolutionized archaeobotany, and Dolores is rewriting the early history of crop plants in the lower latitudes."

A key element behind Piperno's success, her admirers say, has been a determination to plant herself firmly on the scientific side of archaeology, a field that represents an often uneasy marriage between science and the humanities. "Her research in the Latin American tropics, more than that of anyone

Hypothesis tester. Dolores Piperno perfected microscopic methods to trace early agriculture.

else, has put in place hard evidence where there had previously been mainly speculation" about agricultural origins, says archaeologist Dorian Fuller of University College London (UCL).

Philly girl

Piperno, 61, was born and raised in Philadelphia, the child of first-generation Italian immigrants. She first trained as a medical technologist, spending several years looking at blood samples under a microscope in a Philadelphia hospital. With diverse interests in botany and history, she started taking night courses in anthropology. Then, while visiting one of the world's earliest known farming villages in Jericho as a tourist in 1973, Piperno had an epiphany: She suddenly decided to become an archaeologist. "I knew what I wanted to do," she says.

So in 1976, Piperno arrived at Anthony Ranere's archaeology lab at Temple University. Ranere says that at the time, his group's search for early agriculture in the Americas was stalled. He had found a number of prehistoric sites in Panama, some as early as 7000 years old, but little evidence for farming. "We weren't finding any macro plant remains, nor any pollen," Ranere says. "I was kind of at a loss." Piperno recalls saying to Ranere, "Tony, we need another way."

Then Ranere heard a talk by Pearsall, who had pioneered the use of plant microfossils called phytoliths to detect evidence of 5000-year-old maize cultivation in Ecuador. Phytoliths are formed when silica enters the plant from the soil and is deposited within its cells. Because they are mostly inorganic, they remain long after all other traces of plant tissues have vanished. They were well known to soil scientists but not to archaeologists. Pearsall contended that she could distinguish maize phytoliths from those of other plants, as well as from maize's presumed wild ancestor, teosinte, claims that were controversial at the time. "Dolores said, 'This could be a tool for finding these things out,'" says Ranere, who still collaborates with Piperno.

Piperno telephoned Pearsall to find out more about her pioneering techniques. "She called me up and said she was going to do my study all over again in Panama and see if it would work. ... It's common in laboratory sciences to have someone replicate someone else's work but rare in archaeology," says Pearsall.

In 1979, Piperno traveled to STRI in Panama to do her doctoral work on phytoliths

CREDIT: GREG SCHALER

from nearby archaeological sites. She began a lifelong love affair with Panama, eventually landing a staff position at STRI, where she was in residence from 1988 to 2003 and still maintains an active lab today. “It’s a wonderful place,” Piperno says. “I drive 2 hours to a dig site and then start working on the samples the next day in the lab.”

In Panama, Piperno refined and expanded the phytolith technique, putting together a massive reference collection that today includes 2000 species and is used by archaeologists worldwide to classify phytoliths by species, genus, or family. “She defined much of the way we do phytolith research today,” says geoarchaeologist Arlene Rosen of UCL.

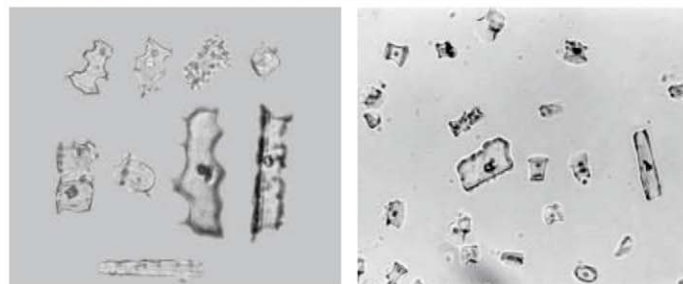
At first, however, many archaeologists were reluctant to accept the early dates Piperno and Pearsall kept finding for maize domestication—at least 7000 years ago in Panama and 6000 years ago in Ecuador. “Dolores took a lot of flak,” Ranere says. “People said these dates couldn’t be possible, it was contamination, things were filtering down into lower archaeological levels.”

Some researchers say there was good reason to be skeptical. At first, Piperno and colleagues “would point to the occurrence of just a handful or less of these microfossils and then to a presumed associated radiocarbon assay and trumpet new antiquity” for domesticated plants, says archaeologist David Browman of Washington University in St. Louis. “I simply found this not to be adequate science.” Browman, along with anthropologist John Staller of the Field Museum in Chicago, Illinois, and others, says that plant roots, worms, and water can easily move the tiny microfossils up or down in archaeological layers. Staller also questions how reliably maize and its ancestor, teosinte, can be distinguished using microfossils. Lately, however, “Piperno and her associates have begun to be a bit more careful” and to put “appropriate controls in place,” Browman says.

Piperno rejects such criticisms. “Soil scientists had [already] established that phytoliths don’t migrate through the kinds of undisturbed soils and deposits that archaeobotanists rely on,” she says, adding that this was confirmed when improved radio-

carbon techniques allowed her and others to date the tiny phytoliths directly (*Science*, 14 February 2003, p. 1054). And she insists that blind tests by numerous researchers have demonstrated that maize and teosinte can be told apart.

For many researchers, the debate over maize origins is now over. “Microfossil evidence has demonstrated conclusively that maize domestication ... took place nearly 9000 years ago and that it was a tropical lowland phenomenon,” asserts archaeologist Mary Pohl of Florida State University in Tallahassee.



Macro and micro. Microfossils can distinguish maize (right hand and lower right) and its wild ancestor, teosinte (left hand and lower left).

Telltale teeth

Piperno’s phytolith research had pushed back the earliest dates for domestication of maize and other plants such as squashes. But many root crops, such as manioc, sweet potato, and arrowroot, leave few identifiable phytoliths. By the late 1990s, Piperno realized that she needed a new tool to solve the entire riddle of agricultural origins in the Americas. Then she read a paper by Australian archaeologists who were identifying taro from its characteristic starch grains. Plants store energy in these microscopic particles, which take on characteristic shapes depending on what plant species produces them. Remarkably, for reasons not entirely understood, they are preserved intact for thousands of years. Food scien-

tists had been familiar with starch grains for decades, but archaeologists were only just beginning to discover them.

To see if starch grains might help reveal the origins of root crops, Piperno and colleagues first looked at stone tools dated as old as 7000 years ago from the Aguadulce rock shelter in Panama and found starch grains from manioc, yams, arrowroot, and maize. They confirmed these findings in 1997, when Piperno and Ranere re-excavated Aguadulce, unearthing more stone tools.

“This was an entire group of plants that we would not know anything about if not for starch grains,” says Pearsall. “The majority of these finds were made by Dolores.”

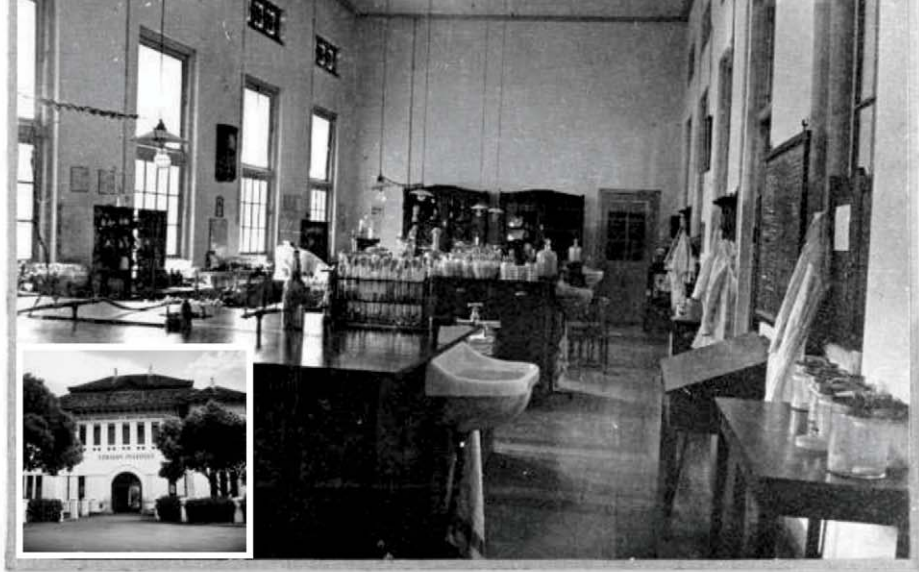
To date, Piperno has built a starch-grain reference collection of more than 400 plant species and used it to explore a variety of prehistoric diets. For example, she demonstrated that people 23,000 years ago at Ohalo II in Israel were grinding wild barley with a grindstone (*Science*, 29 June 2007, p. 1830). And working with archaeologist Tom Dillehay of Vanderbilt University in Nashville, Tennessee, she also found starch grains of cultivated squash, peanuts, and two genera of beans embedded in calculus on human teeth from northern Peru and dated as early as 9000 years ago.

Recently, Piperno realized that this new method could help address another kind of question: How did prehistoric people prepare their plant foods? In a preliminary study, published last year in the *Journal of Archaeo-*

logical Science, Piperno and archaeologist Amanda Henry of George Washington University in Washington, D.C., cooked 10 domesticated crops, including wheat, rice, and lentils, using methods such as boiling, baking, parching, and fermenting, with or without grinding beforehand. They found that many combinations of plants and cooking methods resulted in characteristic starch grain shapes. They’re now applying this work to starch grains from Neandertal teeth, among other projects.

Piperno says she wouldn’t be surprised if these new studies land her in the middle of controversies once again. “All I want to do is continue producing data,” she says. “Eventually, the data win out in the end.”

—MICHAEL BALTER



Glory days. A bacteriology laboratory in the Eijkman Institute (*inset*), circa late 1930s.

HISTORY OF SCIENCE

Righting a 65-Year-Old Wrong

The head of Indonesia's best known science institute was accused of contaminating vaccines and executed by Japanese forces in 1945; new evidence suggests he was innocent

JAKARTA—In July 1944, at a labor camp on the outskirts of Jakarta, several hundred Indonesian forced laborers, or *romusha*, were injected with what was claimed to be a cholera-typhoid-dysentery vaccine produced at an institute run by the Japanese military, which then occupied Indonesia. Within a week, every last *romusha* was dead. A few months later the Kenpeitai, Japan's military police, arrested Achmad Mochtar, director of another research center—the Eijkman Institute—and most of his staff. “They were accused of deliberately contaminating the vaccines as an act of sabotage against the Japanese war effort,” says J. Kevin Baird, director of the Eijkman-Oxford Clinical Research Unit at the present-day Eijkman Institute of Molecular Biology here. On 3 July 1945, with the war in the Pacific nearing its end, Mochtar was beheaded at a Kenpeitai execution ground.

But was Mochtar innocent? Over the past several months, Baird and Mochtar's successor at Eijkman, molecular biologist Sangkot Marzuki, have unearthed evidence that exculpates Mochtar, one of Indonesia's leading scientific lights in the early 20th century. They believe the deaths were the result of a medical experiment by the occupying forces. “It's now well documented that he was made a scapegoat,” says Iris Heidebrink, an archivist at the National Archive in The Hague, Netherlands. Baird and Marzuki plan to rehabilitate Mochtar, with a graveside memorial service on 3 July.

Mochtar was an early star. Born in West Sumatra in 1892, he graduated from STOVIA medical school here in 1916 and

took up an obligatory 2-year posting at a remote clinic in Panyabungan. There he crossed paths with W. A. P. Schüffner, who was carrying out what would become a classic study: the first longitudinal microscopic research on the malaria parasite. “Schüffner was Mochtar's mentor,” says Baird. Perhaps thanks to Schüffner's influence, the Dutch colonial administration in Indonesia—then known as Netherlands East Indies—dispatched Mochtar to the University of Amsterdam for Ph.D. training.

The seeds of Mochtar's demise may have been sown in Amsterdam. His thesis in 1927 largely disproved the widely held belief that leptospira cause yellow fever. The principal promoter of that view was Hideyo Noguchi, a Japanese microbiologist who in 1911 had shown that another kind of spirochete causes the neuropathy of tertiary syphilis. Mochtar returned to Indonesia and continued to work on leptospirosis before joining the country's top biomedical research facility, the Central Medical Laboratory here, in 1937.

It was also here in the late 19th century that the lab's founding director, Christiaan Eijkman, put Indonesia on the scientific map. He won a Nobel Prize in medicine in 1929 for his revelation that a dietary deficiency causes beriberi, a finding that led to the discovery of vitamins. In 1938, the 50th anniversary of its founding, the lab was renamed the Eijkman Institute. Four years later, Japan invaded and immediately interned Eijkman's Dutch staff in camps. (The former Dutch director, W. K. Mertens, died of beriberi in confinement.) Mochtar was appointed director, and the Indonesian staff members carried on their

work. Meanwhile, the Japanese Army commandeered the Pasteur Institute in Bandung, a regional center for vaccine production, and renamed it Boeki Kenkyujo.

Some details of the *romusha* tragedy may never be known. It's unclear, for example, how many *romusha* were injected with the vaccine; estimates range as high as 1500. The laborers came down with symptoms consistent with tetanus, according to three doctors allowed into the camps. “The Japanese described it as a meningitis outbreak, but lumbar punctures ruled that out,” says Baird. The medical team, led by Bahder Djohan, rushed some 90 *romusha* who had not yet become extremely ill to Jakarta's central hospital for antitetanus therapy, but they all died. In the weeks that followed, Eijkman researchers analyzed postmortem tissue samples and concluded that the vaccine had been contaminated with tetanus toxin.

In October 1944, the Kenpeitai arrested Mochtar and his colleagues, as well as central hospital doctors and staff members of the city health service who carried out the vaccinations at the behest of Boeki Kenkyujo. Prisoners later said they had been beaten, shocked, burned, and subjected to waterboarding. “It was brutal and sadistic,” says Baird, who with Marzuki interviewed one survivor and the families of several others, and tracked down written testimony. One doctor died while being tortured, and another died later in captivity.

All the survivors from the Eijkman Institute were released in January 1945 except Mochtar. Later, three independently reported that Mochtar assured them of their release several days beforehand and indicated he would remain imprisoned. “He may have struck a deal with his captors: He would confess to sabotaging the vaccine if his colleagues were released,” says Baird. Mochtar was beheaded on 3 July; according to a Japanese officer's diary, his body was then crushed by a steamroller and his remains were dumped into a mass grave. “Mochtar died a martyr, protecting his subordinates,” says Sjamshudajat Ronokusumo, chair of the Medical Commission of the Indonesian Academy of Sciences.

Baird and Marzuki believe the Eijkman Institute had nothing to do with the vaccine. In a written account after the war, Djohan said he observed vials marked cholera-typhoid-dysentery vaccine produced by Boeki Kenkyujo—the preparation administered to the *romusha*. Boeki Kenkyujo was the only vaccine producer in Indonesia

CREDITS: COURTESY OF EIJKMAN INSTITUTE

before, during, and immediately after the war. “The Eijkman Institute had no vaccine production capacity whatsoever,” says Baird.

There may have been some payback as well for Mochtar having discredited Noguchi’s conclusions about the cause of yellow fever. According to the book *Tales of a Revolution* by Abu Hanifah, an independence fighter and Mochtar’s nephew, when the Kenpeitai searched Mochtar’s home after his arrest, the only item they took was his thesis challenging Noguchi.

Baird and Marzuki knew that a clearer picture of the circumstances surrounding the *romusha* vaccinations would be essential to clearing Mochtar’s name. They had to uncover the motive. Conditions in the labor camps generally were deplorable; only one in five of the estimated several million *romusha* survived the experience. “Why vaccinate people you do not even bother to feed?” asks Marzuki.

Heidebrink offers one possible explanation. The tragedy occurred at Klender, a *romusha* camp touted as a “model village.” “They were having trouble getting enough *romusha*, so to help with recruitment they built this village,” says Heidebrink. Families were relocated to Klender and after a few weeks, men were sent to work in the far reaches of the archipelago; wives and children would remain behind and, among other benefits, had access to health care, Heidebrink says. She believes that the *romusha* deaths were “a terrible mistake,” most likely stemming from contaminated

and a facility as experienced and sophisticated as Boeki Kenkyujo would not screw this up,” says Baird. They believe the tragedy at Klender was a medical experiment. “This was something more sinister than we expected when we first started to look into it,” Marzuki says. Getting to the truth is not easy: With the end of the war nigh, “the Japanese military destroyed all documents, as far as we know,” says Heidebrink. And many Dutch archives were destroyed during Indonesia’s war for independence in the late 1940s.

As circumstantial evidence, Marzuki points to another episode documented by an Australian war crimes tribunal in 1951. In January 1945, the fleet surgeon of the Japanese Navy’s Second South Seas Expeditionary Fleet, Hirosato Nakamura, “was turning over every stone to get his hands on antitetanus plasma. He needed the stuff desperately,” Baird says. None could be spared by the Army facility in Bandung. An alternative would be tetanus vaccine, which uses inactivated toxin, or tetanus toxoid. “If you have tetanus toxoid, you don’t need plasma; problem solved,” Baird says. Nakamura injected 17 condemned Indonesian prisoners with a tetanus vaccine whipped up by the military. The prisoners tolerated the vaccine well. To test efficacy, Nakamura injected them with pure tetanus toxin; 15 died, and the two survivors were executed. The Australian War Crimes Court convicted Nakamura of unlawful killing and sentenced him to 4 years in prison.

That incident suggests a possible motive for the apparent vaccinations of the *romusha*: With antitetanus plasma and vaccine so scarce in the waning months of the war, the vaccinations could have been a challenge with tetanus toxin to gauge the efficacy of an experimental vaccine, Baird and Marzuki suggest. “The probability of accident may be considered so remote as to be virtually impossible,” Baird says.

After the war, the Eijkman Institute lost its luster. “What happened to Mochtar and



To rest in peace. A 3 July graveside service aims to rehabilitate Achmad Mochtar (inset), unjustly accused of killing hundreds of Indonesians during World War II.

the subsequent trauma during the independence war affected the institute significantly,” Marzuki says. After a long decline with no effective leader, Eijkman was shuttered in 1965. Then in 1992, after watching how Singapore and other Asian neighbors were developing biotechnology industries, B. J. Habibie, the longtime science minister who later briefly served as the country’s president, recruited Marzuki from Monash University in Melbourne to lead a revival of the Eijkman Institute of Molecular Biology. After a half-century hiatus, Mochtar had a successor.

Indonesian scholars have long felt that Mochtar suffered an injustice. Marzuki says that for years he quietly compiled a dossier on his predecessor. “I knew it was important to clear his name,” he says. Baird was the catalyst. His interest in the case was sparked earlier this year, after he asked a student to write a history essay to help improve her English. The essay glossed over the war years. “She said she didn’t know anything about it. I didn’t either,” Baird says. He began reading up. Chatting with Marzuki, he learned about Mochtar. “I was hooked; I had to find out more,” Baird says.

In addition to amassing a trove of documents and interview records, the duo early last month learned about the location of Mochtar’s grave. That discovery inspired the memorial service, which will be a brief, low-key affair for a few dozen of Mochtar’s family and Eijkman staff members. The quest for additional evidence to restore Mochtar’s reputation—and reveal what really happened at Klender in 1944—will continue.

—RICHARD STONE



Searching for the truth. J. Kevin Baird (left) and Sangkot Marzuki contend that the *romusha* deaths were a sinister experiment.

vaccine. Indeed, Aukje Zuidema, a historian at the Dutch Institute for War Documentation in Amsterdam, notes in *The Encyclopedia of Indonesia in the Pacific War* (2010) that the wartime head of Jakarta’s health service suspected that the typhus-cholera-dysentery vaccine contained “virulent bacilli” due to “less accurate production methods.”

Marzuki and Baird are dubious. “Very simple guinea pig testing ensures that no contamination occurs in vaccine production runs,

LETTERS

edited by Jennifer Sills

Racial Differences in Breast Cancer Patterns

THE DEBATE OVER THE CHANGE IN MAMMOGRAPHY RECOMMENDATION FROM THE U.S. Preventive Services Task Force (“Brawling over mammography,” E. Marshall, News Focus, 19 February, p. 936) has helped highlight the gap in research and technology. The recommendation, however, would benefit from more consideration of racial differences in breast cancer incidence patterns. Seewaldt (1) noted that current mammography recommendations were primarily based on randomized trials of Caucasian women. However, the breast cancer mortality rate is higher and mammographic density is typically lower in African-American women than in Caucasian women. Similarly, observations since the 1970s have shown that breast cancer incidence peaks between 40 and 49 in Asian and Asian-American women before decreasing at menopausal age (2–8). It seems risky to simply recommend against routine mammography between 40 and 49, regardless of racial differences.

LU SHI

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Cryptic Loss of India's Native Forests

INDIA SUSTAINS SOME OF THE WORLD'S MOST imperiled forests, which encompass parts of three recognized biodiversity hotspots (1). How are these crucial forests faring? In 2009, the Forest Survey of India (FSI) announced ostensibly good news: Indian forests had expanded by nearly 5% over the preceding decade (2). Unfortunately, as scientists who have long studied Asian forests, we believe that this view is misleading.

First, local studies reveal that native Indian forests, such as those in the Western Ghats (3), Eastern Ghats (4), and Himalayas (5), have declined rapidly in recent decades. Second, in its automated analyses of satel-

lite imagery, the FSI lumps together all forest types. Native forests are pooled with exotic tree plantations, such as eucalyptus, acacia, rubber, teak, or pine trees, which have very limited value for endangered biodiversity (6). Since the early 1990s, plantations in India have expanded rapidly, at a reported mean rate of ~5700 km² (7) to ~18,000 km² (8) per year, with the higher value being more credible. If one subtracts plantations from total forest cover (2), then India's native forests have actually declined at an alarming pace, from 0.8% to 3.5% per year. The biggest driver of this decline is forest cutting for fuel wood, with an estimated 94.6 million metric tons of fuel wood being consumed annually (9).

The cryptic destruction of India's native

forests highlights a key challenge for those attempting to understand current trends in forest ecosystems. Data from satellites are increasingly used to monitor changes in net forest cover [e.g., (2, 7)], but such estimates typically lump together native, exotic, and degraded vegetation. A failure to discern such changes could paint a highly misleading picture of the fate of the world's native forests.

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Variability of Our Somatic (Epi)Genomes

A MOTTO OF THE HUMAN GENOME PROJECT could have been “everything that happens in the world depends on [DNA] sequences” (1); the Project's quasi-completion was solemnly celebrated in 2000. Still, in 2010, chief-genomicists Francis Collins and Craig Venter reportedly admit that its impact on medicine was “not much” (2).

The human genome seems more complex but less autonomous than originally believed: To function, it often has to modify

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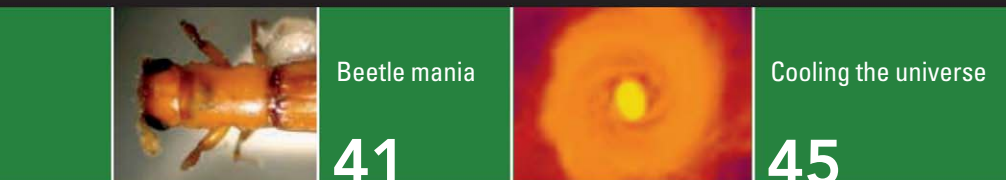
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specific bases of its DNA and interact with many proteins and RNA, originating the epigenome. In the process, the interactors themselves may change.

At conception, the parental nuclei fuse in the zygote, which after three to four mitoses gives ~10 blastomeres, often aneuploid (3). These and/or their descendants are quickly shed, whereas the normal cells are channeled along our ~250 somatic lineages. Thus, the zygotic genome is really ephemeral: Over time, it creates trillions of cells unfolding their phenotypes as dictated by genomic sequences and epigenomic marks. The latter may contribute to the rearrangement of the former through, for example, reverse transcription and transposition. Somatic genome variations creep in, aided by elusive environmental inputs and well-documented programs (such as the immune response). Each somatic cell suffers ~10⁴ lesions per day (4): Because this damage is repaired in different ways, hundreds of singularities haphazardly alter the genome of the various cells. This eventually may corespond to the persistence of gaps in their finished sequences (5).

Therefore, the dogma that all the cells of an individual contain the same DNA needs revision (6). If so, we must ask: Which is our real genome, and how safely and efficiently can we induce back its embryonic or even zygotic potential (7)?

The return of epigenomics recalls Miescher's nuclein, which later became Flemming's chromatin and now may be known as the (epi)genome. To gauge its putative tissue- and stage-variability, we

need more flexible conceptual approaches and sharper analytical tools. Together, they should enable us to fully digitize our (epi)genomes, with the caveat that their analysis would be generally destructive. The variability of our somatic (epi)genomes would make each of us a mosaic: The tesserae of this mosaic are our trillions of cells, each graced with its own (epi)genome and life span (8).

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FDA Regulations for Drug Development

THE EDITORIAL "CANCER THERAPY REFORM" by Genentech Chairman A. D. Levinson (9 April, p. 137) highlights possible confusion about U.S. Food and Drug Administration (FDA) regulation of developing therapies. Contrary to Levinson's claims, there is not now, nor has there ever been, a regulation (or regulations) that prohibits, or even impedes, the development of two new molecular entities in combination.

CORRECTIONS AND CLARIFICATIONS

Reports: "Bose-Einstein condensation in microgravity" by T. van Zoest *et al.* (18 June, p. 1540). J. Reichel's affiliation was listed incorrectly. Affiliation 9 should be: Laboratoire Kastler-Brossel de l'Ecole Normale Supérieure, Université Pierre et Marie-Curie-Paris 6, CNRS, 24 rue Lhomond, 75005 Paris, France.

News Focus: "Ever-smaller lasers pave the way for data highways made of light" by R. F. Service (14 May, p. 810). The credits for the first two figures were reversed. The credit should have read, "Credits (top to bottom): M. T. Hill *et al.*, *Optics Express* **17**, 13 (18 June 2009); M. Nezhad *et al.*, *Nature Photonics*, published online 18 April 2010." The credits have been corrected in the HTML version online.

News Focus: "In the shadow of Jane Goodall" by J. Cohen (2 April, p. 30). In the graphic on page 31, Site 13 was misspelled, as was the affiliation of lead researcher Kevin Hunt. It should have read Semliki-Toro Wildlife Reserve, Uganda, and Hunt works at Indiana University.

Research Articles: "Formation of ArF from LPdAr(F): Catalytic conversion of aryl triflates to aryl fluorides" by D. A. Watson *et al.* (25 September 2009, p. 1661). The permanent address for M. Su should have been listed as Singapore-MIT Alliance, E4-04-10, 4 Engineering Drive 3, 117576 Singapore.

The FDA is uncertain as to the source of this confusion but believes that additional clarity is warranted and, thus, plans to issue clarifying guidance on the matter. The current regulatory schema permits precisely what Levinson advocates, and we concur that such an approach is exactly what is needed, under appropriate circumstances, in this era of increasing ability to discern specific molecular pathways, particularly for cancer and infectious diseases.

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Response

I THANK BEHRMAN AND WOODCOCK FOR their Letter. Woodcock acknowledged the ongoing confusion in her Brookings' presentation of 14 September 2009 (1), in which she stated that the combination rule is widely misinterpreted, to the extent that the FDA would be developing guidance on the topic. Further clarification of the combination rule as it pertains to non-fixed drug combinations is urgently needed to support innovative drug development. Genentech is encouraged by the FDA's 8 June 2010 Federal Register request for comment on the codevelopment of investigational drugs (2)—an important step in the development of guidance. The Behrman and Woodcock Letter highlights the importance of decreasing real and/or perceived barriers to bringing novel combination therapies to patients with serious and unmet medical conditions.

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

SCIENCE TOURISM

Some Stops for Summer Trips

Because we suspect that you already have an ever-growing stack of unread books, this year we offer some different suggestions for summer escapes: a few exhibits, events, and sites for science-minded travelers. The following is a very small sample of such destinations, and recommendations for future coverage are most welcome (science_bookrevs@aaas.org). If you won't be traveling this summer or are otherwise still looking for reading material, you may wish to check our summer reading ideas from past years: e.g., *Science* 324, 2167 (2009), *Science* 316, 1845 (2007).

—Sherman J. Suter

Reflecting Berlin, Past and Present

Olafur Eliasson: Innen Stadt Außen. Daniel Birnbaum, curator. Martin-Gropius-Bau, Berlin. To 9 August 2010. www.gropiusbau.de

WeltWissen [Universal Knowledge]: 300 Years of Science in Berlin. Martin-Gropius-Bau, Berlin. 24 September 2010 to 9 January 2011. www.gropiusbau.de

Berlin is a city of contrasts, with landmarks that reflect depraved dictatorships and peaceful revolutions, Prussian order and anarchist chaos—sometimes all on a single street corner. Two intriguing exhibits this summer and autumn offer visitors a chance to see the city in a new light.

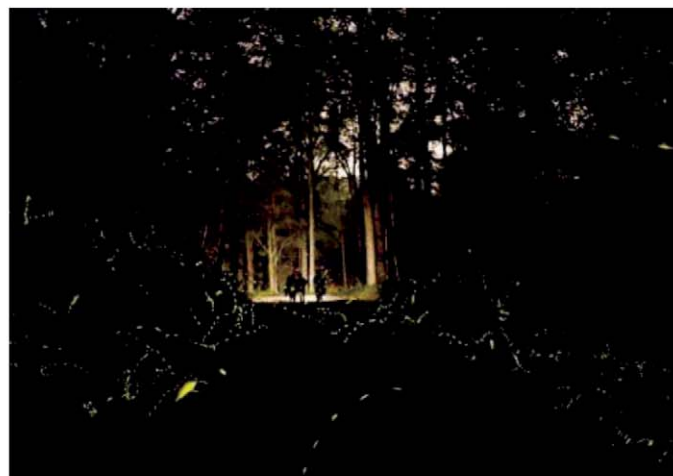
Playing tricks with light, reflections, and human perception is a trademark of the Danish-Icelandic artist Olafur Eliasson. His latest show, in his adopted hometown, is no exception. The exhibit, *Innen Stadt Außen*, began last winter with more than a dozen temporary works scattered throughout the city. These included bicycles with mirrored wheels that seemed to melt into their surroundings as well as logs, collected from the coast of Iceland, that were placed across intersections and pedestrian walkways. (The exhibit's title is a pun: Literally "Inner City Outside," it also sounds like "inside instead of outside.")

The main installation, at the Martin-Gropius-Bau, starts playfully, with curved mirrors and multicolored shadows. But then the visual tricks get more intense and become more intriguing for enthusiasts of optics and visual perception. One room seems to turn the world into shades of gray. A film shows a truck with a huge mounted mirror driving through Berlin, reflecting and bending the cityscape onto itself. A mirrored hall, titled *Microscope*, can make it appear as if one is standing on top of the museum's famous glass portico. The final piece draws the visitor through

a rainbow-colored fog that is disorienting enough to warrant a warning for claustrophobics.

For visitors who don't arrive until later in the summer, one outdoor installation, *The Blind Pavilion*, will remain at the Pfaueninsel, an island in Wannsee Lake in southwest Berlin, until 31 October. By then, the Martin-Gropius-Bau will be hosting an exhibit reflecting another side of this multifaceted city: *WeltWissen: 300 Years of Science in Berlin*. Berlin is currently celebrating a "Year of Science" in honor of multiple anniversaries: The 300th of both the Berlin-Brandenburg Academy of Sciences and the Charité Hospital, one of Europe's largest research hospitals; the 350th of the city's library, the Staatsbibliothek; the 200th of the Humboldt University; and the 100th of the founding of the Kaiser Wilhelm Society, the forerunner of today's Max Planck Society. *WeltWissen* will spotlight scholars who have lived and worked in the city, such as explorer and biologist Alexander von Humboldt, Albert Einstein, Fritz Haber (whose work on ammonia synthesis revolutionized agriculture), and Gerhard Ertl (the 2007 Nobel laureate in chemistry). Its coverage does not ignore less-welcome discoveries (e.g., Haber's World War I work on chemical weapons). And the exhibit will also examine lows and highs of scientific research in the city, from the Nazi-era persecution and expulsion of scientists to the postwar attempts to rebuild science both east and west of the Berlin Wall.

—Gretchen Vogel

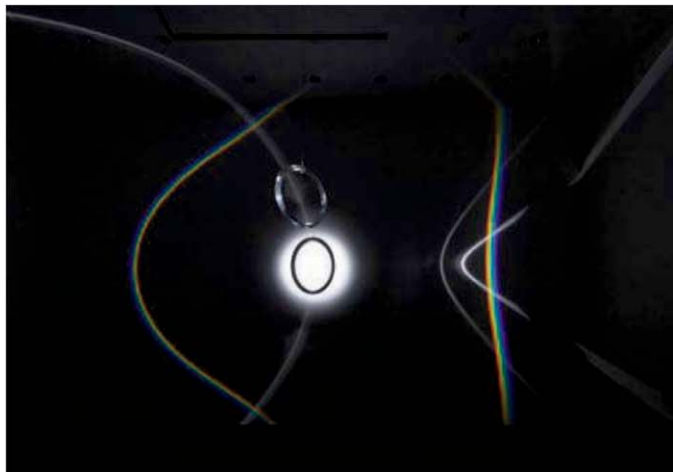


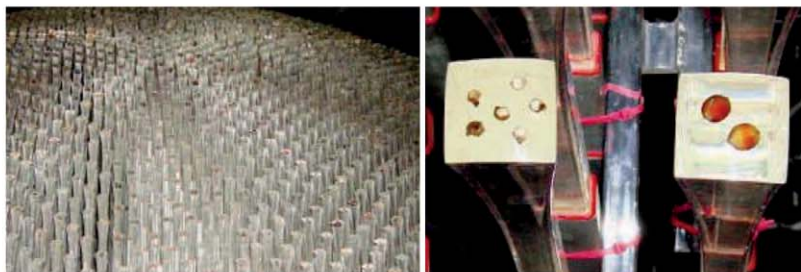
Science After Dark

Synchronous Fireflies. Great Smoky Mountains National Park, Tennessee. www.nps.gov/grsm/naturescience/fireflies.htm

The event is not really a festival, it's not at a museum, and you have missed it this year. Despite all of that, during the second week of June, Great Smoky Mountains National Park provides a scenic stage for the displays of the synchronous firefly, *Photinus carolinus*. The males gather in groups whose members synchronize their flashing lights; how this concerted effort pays off with the females remains unclear. Fireflies, which like a damp environment, can be seen throughout the park, but most visitors view them on the Little River Trail, starting at the Elkmont campground. On evenings during the synchronous firefly season, visitors to the site must use the trolley service from the Sugarlands Visitor Center, just south of Gatlinburg. Several hundred people can be accommodated each night, but the parking lot can fill (especially on weekends), so you may want to arrive early. However, the firefly action will not start until after sundown (around 9 p.m.); if you come early, plan on enjoying other aspects of the park. The rangers recommend a flashlight covered with red cellophane, and only a heavy rain stops the show. If the night is clear, bring a star chart and binoculars, as the night sky in the Smokies is wonderfully dark.

—Phillip Szuromi





The Largest World's Fair

World Expo 2010. Shanghai, China. To 31 October 2010.
<http://en.expo2010.cn/>

The more than 200 pavilions at the current world's fair in Shanghai offer countless fun and science-related exhibits, unique multidimensional movies, and interesting activities. These range from ancient fossils (including 125-million-year-old flowers, early flying birds, and dinosaurs) to futuristic, cutting-edge sound, lighting, and sensor technologies that stir the imagination. Visitors can join interactive multimedia programs and enjoy spectacular views and outdoor performances. Exhibitors comprise 189 countries, 57 international organizations, and each of China's 31 provinces—municipalities—autonomous regions.

The Expo's theme, "Better City, Better Life," underscores the goal for environmental and socioeconomic sustainability in our rapidly urbanizing world. Reflecting the theme, the Expo displays numerous innovative ideas and emerging technologies that promote higher efficiency, lower CO₂ emissions, less waste, and the renewable use of energy and other resources. One example is the United Kingdom's visually awe-inspiring "Seed Cathedral," with 60,000 swaying fiber optic rods (filled with seeds, which will later be planted) that provide interior light during the day and become an enormous glowing piece of art at night. Another is on display in the Chinese national pavilion: the world's first "carbon negative" concept car, which would run on renewable energy and emit oxygen.

The first world's fair to be hosted by a developing country, Shanghai 2010 is expected to draw a record 70 million visitors. The most popular pavilions require reserved tickets and patient waits in dragonesque lines. Those unable to get away to Shanghai may enjoy virtual tours of the fair at <http://en.expo.cn.>

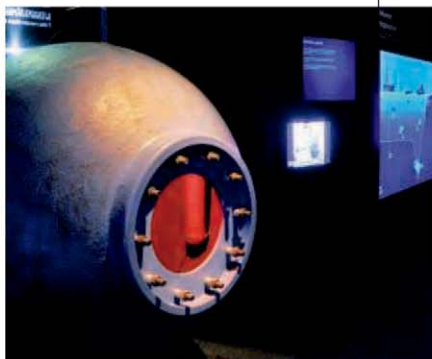
—Jianguo Liu and Shuxin Li (Michigan State University)

Sink into the Depths of the Sea

The Deep. Natural History Museum, London, in collaboration with Natural History Museum, Basel, and the Senckenberg Research Institute and Nature Museum, Frankfurt am Main. To 5 September 2010.
www.nhm.ac.uk/thedeep/

In the pitch-black water, 3 km down, under crushing pressure, food is problematic. So when a whale falls to its death, it's a bonanza for abyssal creatures. At the center of the exhibition *The Deep* is a partial skeleton of a sperm whale, used to illustrate what happens after a succession of scavenging hagfish and crustaceans have done their work. Next it's the turn of the snotworms (*Osedax*), recently discovered annelids that burrow into whale bones and, with the help of symbiotic bacteria, digest away the final traces of the behemoth. No wonder fossil whales are very rare.

In the gloom of the exhibition, projections of self-illuminating life forms swim around models of a giant squid and a sperm



whale hanging from the ceiling. There's a case of giant radiolarians, miraculously magnified in blown glass by Leopold and Rudolf Blaschka, who are famous for the exquisite collection of glass flowers commissioned by Harvard University's Museum of Natural History. Round the corner is a replica of William Beebe's bathysphere; if you put your head inside, you will hear the voices of the pilots describing the constellations of flashing lights they saw emitted from myriads of unexpected animals. And there are

indeed nightmare monsters, as the arrays of bizarre pickled fish attest to, with their huge eyes, glowing lures, and extensible jaws adapted to surmount the difficulties of snaring a morsel in the dark cold. Sailors imagined mermaids and krakens populated the deep; we know little better now, even of creatures as huge as giant squid. Despite the best efforts of ocean explorers, research in the deep is difficult and expensive; we learn that the crew of the *Challenger* voyage (1872–1876) complained mightily of the relentless tedium of trawling and hauling nets on their global survey. This exhibition supplies visitors with the chance to taste deep-ocean travel without any risk of dampness. You can step inside the submersible *Mariana*, take the captain's seat, and watch the endless snowfall of dying surface organisms drifting across a video porthole. Don't stop here though: Dip into *The Deep* to cool off for an hour, and then make your own voyage through the multitudes of extraordinary natural wonders and many spectacular exhibits the museum has to offer.

—Caroline Ash



Once Upon a Time on an Ancient Reef

Geikie Gorge National Park. Western Australia. www.dec.wa.gov.au/component/option,com_hotproperty/task,view/id,43/Itemid,1584/

Scenic Geikie Gorge, in the rugged Kimberley region of Western Australia, offers visitors two journeys back in time. As the National Parks boat trip winds its way upstream, the towering cliffs of white and buff-gray limestone will whisk them back to the Devonian period, around 380 million years ago, when most of northwestern Australia was flanked by a reef system almost the scale of the living Great Barrier Reef. Geologists hail the gorge as one of the most spectacular exposures of rocks this age anywhere. Myriad bizarre life forms once flourished on, in, and around this fossil reef. It was built up not by coral but by algal communities called stromatolites aided by layered, sponge-like stromatoporoids.

The Devonian reef exposed along the Fitzroy River runs for 325 km across the Kimberley landscape, in remote places graced by the giant bottle trees (boabs). Just south of Fitzroy Crossing (the town closest to the gorge), jagged outcrops of the reef are expressed as sharp, linear mountain ranges, albeit low ones. Here some of the world's best fossil fishes can be found as three-dimensional, uncrushed skeletons entombed in limestone concretions that litter the valley floors for 80 or so km. In his 1979 series *Life on Earth*, David Attenborough traveled here (to a cattle ranch called Gogo) to teach us about fish evolution. Expeditions I have led to the site over the past 25 years have been fortunate to stumble on some interesting discoveries: an armored placoderm fish (a long-extinct group) that held an unborn embryo inside her, still linked by a mineralized umbilical cord; a fish specimen with

the oldest complete male mating structures, intact; a lungfish that revealed to us how fish first began to breathe air, while still living in marine conditions. The Gogo has been instrumental in refining our view of vertebrate evolution, as so much of the human body plan first developed in these early fish.

Where the boat turns around to head for home, visitors can glimpse green patches of dense, vine-thicket rainforest between the cliffs of pale rock. Trees cling to rocky ledges, their slender tap roots at times penetrating 20 m of solid rock to reach the water underneath. There is a huge wealth of wildlife to be seen, heard, and at times encountered unexpectedly. In spring, you might be alarmed by the sound of thousands of large screeching fruit bats ("flying foxes"). All year round, 160-cm-tall brolga cranes stride in the shallows, keeping a wary eye on groups of toothy, narrow-muzzled, freshwater crocodiles that sun themselves on sandy banks and fallen logs. If you stay overnight at a lodge near the river, you will surely be woken by a magnificent dawn chorus of almost-deafening bird calls.

The second journey the visitor takes here is back to a time before Europeans came to Australia, to the dreamtime land of the Bunaba people. Their rock paintings adorn caves throughout the region, and guides from the tribe can take you on a tour to demonstrate the abundant wealth of native foods in the region. The Fitzroy River links the ancient, the present, the cultural, and the spiritual themes of the landscape. One can truly learn many lessons from the past here.

—John Long (Natural History Museum of Los Angeles County)



Facing Higher Water

Rising Currents: Projects for New York's Waterfront. Museum of Modern Art, New York. Barry Bergdoll, curator. To 11 October 2010. www.moma.org/visit/calendar/exhibitions/1031

By 2080, climate change may deliver a 60-cm rise in sea level along with more frequent and more violent storm surges to New York City's waterfront. *Rising Currents* (the first of five exhibitions in the series "Issues in Contemporary Architecture") presents five proposals for reconfiguring the area's shores to adapt to such a threat. Each multidisciplinary team of architects, engineers, and landscape designers addressed a different terrain—from the low-lying landfill areas around Bayonne and Jersey City to the heights on either side of the Verrazano Narrows bridge. Their solutions are depicted in models, maps, and drawings as well as described in videos by the teams. In one concept, existing piers and warehouses are transformed into a recycling facility to convert discarded glass into building blocks for a glass reef. The reef would act as a breakwater and home for marsh grasses and marine life. Another group envisioned the polluted area around the Gowanus canal converted into a vast weblike structure that would support an oyster farm. A third would deal with storm surges by having buildings hanging from a shared bridge. Rather than fighting climate change, the teams envisioned infrastructures that exploit altered conditions to reimagine what an urban environment can provide for its inhabitants.

—Barbara Jasny

A Sporting Chance

Athletes and Science. Le Musée Olympique, Lausanne, Switzerland. To 13 March 2011. www.olympic.org/en/content/The-Olympic-Museum/

Walking under a high-jump bar set at the long-standing world record of 2.45 m makes it clear that the Olympic Museum celebrates the remarkable feats of athletes. Therefore, it is no surprise that the museum's new *Athletes and Science* exhibition projects a somewhat guarded view of science's role in sports. As visitors enter, a cautionary message proclaims that despite the advantages science and technology provide, "sport must remain human."

Along those lines, the exhibition suggests that science and technology may aid the supporting participants in sports as much as they help the athletes themselves. For instance, coaches have tools that track player movement in team sports, trainers count on state-of-the-art physiological monitoring to improve performance, referees rely on technology to obtain unambiguous results from photofinishes, and judges use technology to eliminate bias from subjectively evaluated sports such as figure skating. As to what the athletes stand to gain (or lose), the exhibition does not discuss perhaps sports' largest concern—doping—because the museum itself houses a permanent, surprisingly open and apprehensible exhibition on that topic.

Unfortunately, the exhibition generally fails to distinguish technological advancements (such as touch-pad sensors and stop-motion cameras) from scientific research. There are, however, a few short presentations on topics such as how muscles twitch, how different materials absorb shock or propagate vibration, and the basics of hydrodynamics. Whereas the exhibition's general theme—that science and technology deliver both benefits and harms to sports—is clear throughout, there is only a brief mention that sport provides a "fabulous research opportunity for science" or researchers.

One futuristic display portrays an Olympic Games (2150) in which the world of sport has become too reliant on technology: A swimmer sets a new world record over three times as fast as the current mark thanks to both a "polymeta-superflottanyl" bodysuit that dynamically controls muscle movement and a rigorous course of gene therapy that increased the surface area of hands and feet. Tennis players face missile-like serves that are aided by cylindrical carbon nanotube implants in their opponent's arms. At least such "advances" have not been incorporated in any of the exhibition's several interactive displays. Instead, visitors can explore how oxygen is absorbed in the blood (by riding a stationary bike in a low-oxygen chamber to simulate high elevation bicycling) and how our brains control our bodies' reaction times (by timing how long it takes to come out of sprinters' starting blocks).

—Nicholas S. Wigginton

Lions, Tigers, Bears, and Much More

San Diego Zoo. San Diego, California. www.sandiegozoo.org/zoo/

Not far from downtown San Diego, one can view a great variety of animals and learn about biological diversity, ecology, evolution, and conservation, all while having fun. The San Diego Zoo hosts "charismatic megafauna" such as elephants, lions, tigers, polar bears, bonobos, and California condors. Its 40-hectare site in Balboa Park also houses hundreds of other species of mammals, birds, reptiles, amphibians, and insects. Additionally, the grounds double as a botanical garden and boast an impressive collection of plants (many of which are rare), including acacias, bamboos, cycads, bromeliads, figs, palms, and orchids. For younger visitors, there are animal shows and a children's zoo (with petting area and playground). My sons particularly enjoyed the zoo's Lost Forest zone, as it highlights many of the large animals.





Education and conservation are major themes, both at the exhibit level and behind the scenes. Many exhibits offer details about ecology, behavior, adaptations, and evolutionary history. The biologist and anthropologist in me are pleased that the Monkey Trail exhibits (which include three of our four closest living relatives, missing only common chimpanzees) provide a phylogenetic perspective on primates and humans' place within them as well as information on aspects of primate communication and anatomy.

The zoo is famous for its work with giant pandas, and watching the newest cub romp around delights kids of all ages. Displays explain which species are threatened and what visitors can do to help. As an example, the zoo facilitates cell-phone recycling to reduce the demand for coltan, a rare metallic ore obtained from central Africa (where increased mining has led to habitat loss and increased poaching). The Elephant Odyssey area does a particularly nice job integrating a historical perspective on extinct animals from the Pleistocene of Southern California (mammoths, American lion, sabertooth cat), their living relatives (elephants, lions, jaguars), and the zoo's ongoing efforts to study and save modern elephants in Africa (Project Elephant Footprint). By placing its animals in context, the zoo helps us to better understand them and the world around us—and to contemplate how bleak the future would be if we lost them.

—Tasha Altheide (University of California, San Diego)

Magical Gadgets from Manga

Doraemon's Scientific Future. Nippon Kagaku Miraikan [National Museum of Emerging Science and Innovation], Tokyo, Japan. Through 27 September 2010. www.miraikan.jst.go.jp/en/spevent/doraemon/

One of the classic Japanese Manga cartoons features a cat robot, Doraemon, from the future. Children, the cartoon series' primary viewers, are fascinated about the amazing gadgets Doraemon introduces to help out his friends when they are in need. When I was growing up, one of my favorite inventions from the show was the "Takecopter," a special propeller you attach on top of your head to fly.

The Doraemon exhibition at the Miraikan showcases 25 of the most creative, advanced, or promising technologies, such as an "invisible cape" using metamaterial, medical microrobots, and a nodding and talking robot. Each display describes the usefulness of the technology and the challenges of developing it. Each also introduces the researchers behind the invention. In fact, many of those scientists are also big fans of the cartoon. They have been inspired by the technological possibilities depicted in the show and are contributing to the advancements that may actualize these devices. With my attraction to the Takecopter, I enjoyed the GEN H-4, the world's smallest helicopter (visitors can pose in its seat). The fascinating invisible cape



seems normal but is made from metamaterial that bends light rays. The state-of-the-art science and technology presented in the exhibit should stimulate curious minds of adults and children alike.

—Satomi Maeda (Leave a Nest Company, Tokyo)

When the Universe Was the Size of a Football

Evolution of the Universe. Deutsches Museum, Munich, Germany. Through December 2011. www.deutsches-museum.de/en/exhibitions/natural-sciences/astronomy/exhibition/cosmology

How did the universe begin? How do galaxies, stars, and planets form and evolve? What is the universe's ultimate fate? These are some of the foremost questions cosmologists are trying to answer, and they underlie the Deutsches Museum's exhibition *Evolution of the Universe*. Put together by five leading local research institutions, this Munich exhibition presents a snapshot of modern cosmological knowledge, organized as a journey through time from the Big Bang (13.7 billion years ago) through to the present day.



After finding their way through the museum (one of the world's largest science and technology museums) and through the several floors of the permanent (and quite comprehensive) astronomy displays, visitors arrive at a small circular room. In its center stands the Universe Cinema—a round purple couch from which a 10-minute video is projected onto the ceiling. It will be tempting to lie down and simply gaze at the beautiful images, perhaps even taking off your shoes and relaxing, as some people did the day I visited. But don't leave before exploring this exhibition that invites you to think and to use your imagination. Through printed text and images, interactive screens, models, and hands-on demonstrations, you will be introduced to complex concepts such as cosmic inflation and gravitational lenses.

The matter-antimatter asymmetry in the universe, for example, is imaginatively illustrated with two boxes of colored sand. One, containing a billion grains of sand, represents antimatter; the other, with one extra grain, represents matter. After pairs of particles and antiparticles annihilated one another in the early universe, one extra particle remained for each billion pairs, the surplus of matter from which everything in the universe is made. The origin of this asymmetry remains unknown. In another intriguing demonstration, a box filled with metal spheres being shaken over hidden magnets demonstrates how dark matter determined the large-scale distribution of luminous matter over cosmic time.

Some demonstrations are more effective than others, and the quality of the images in the Universe Cinema could certainly be improved. Nonetheless, I enjoyed the exhibition's noncondescending tone and, above all, the fact that visitors are exposed to questions that are being actively researched—some in research centers not far from the museum.

—Maria Cruz

10.1126/science.1193490

Synthetic “Life,” Ethics, National Security, and Public Discourse

Mildred K. Cho^{1*} and David A. Relman^{1,2}

The work by Gibson and colleagues in this issue of *Science* (1) showcases technological achievement, highlights the promises of science, and raises questions about the nature of life. The ensuing discussion has featured a number of concerns about biosecurity and ethics—some real, some imagined.

“Synthetic genomics” refers to the laboratory synthesis and assembly of genomes and their expression to produce viruses or cellular life forms, whereas “synthetic biology” refers more broadly to the creation of synthetic biological systems that are programmable, self-referential, and modular. The design of synthetic genomes has been largely based on the sequence of known, naturally occurring genomes. Until now, because of the challenges of synthesizing and assembling large pieces of nucleic acid accurately and of choreographing genome replication and expression properly, only viral genomes have been synthesized and expressed. However, the associated skills (i.e., to read, write, and assemble DNA) have advanced considerably over the past several decades (2). This rapidly changing landscape also includes other (non-synthesis-based) genetic engineering techniques for generating, expressing, and screening novel genetic diversity, such as directed molecular evolution and DNA shuffling. What has lagged behind most noticeably is a predictive understanding of function and of the emergent properties of cells based on genome sequence and, hence, the insight necessary to design truly novel forms of life.

The kinds of individuals participating in the life sciences revolution have also expanded to include nonprofessional scientists and those trained in disciplines well outside the traditional mainstream (3). The public sees a growing population of operators with various interests in manipulating and controlling life, but less well articulated rationales.

In the midst of revolution, and especially over this past decade, awareness of risk in the life sciences has become heightened, in part

because of the growing and more widely disseminated capabilities mentioned above and in part because of a less-stable global political and social landscape (4). The greatest challenge in addressing biosecurity and ethical concerns has been, and will be, to design effective oversight mechanisms that avoid undue harm to the overwhelmingly beneficial life sciences enterprise.

The U.S. National Science Advisory Board for Biosecurity (NSABB) has proposed a definition for dual-use research of concern and a framework for overseeing this research, including approaches for outreach, education, and risk communication (5). The NSABB criterion is purposefully broad and defines dual-use research of concern as “research that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health, agriculture, plants, animals, the environment, or materiel” [(5), page 1, footnote 1]. In addition, NSABB and other organizations have offered recommendations for managing some of the risks associated with synthetic genomics, prompting preliminary governmental response (6). [For example, the U.S. government is developing guidance to producers of synthetic genomic products regarding the screening of orders for sequences of “select agents and toxins” (7)]. A major unresolved problem is the increasingly dangerous reliance on microbial threat lists, and another is the arbitrariness of microbial taxonomy based on sequence homologies (8). Microbial genetic diversity discovered in nature, as well as generated in the laboratory, increasingly blurs these taxonomic boundaries. Lists of named agents create an illusion of having defined the spectrum of potential threats.

From a security perspective, Gibson *et al.* do not cause particular concern. Although synthetic genomics poses potential risks and will increasingly do so in the future, the methods and findings here are probably of limited applicability and generalizability (e.g., limited to genomic transplantation of cell-wall deficient organisms), and may be difficult to apply to other kinds of organisms. In addition, the work does not provide new guidance

Synthetic biology draws notice to the need for balanced and informed discussion about benefits and risks in the life sciences.

or instruction that aids in the creation of an organism with new worrisome attributes. In 1999, Cho *et al.* (9) identified ethical issues associated with efforts to create synthetic genomes and synthetic life, including the problems with reductionist approaches and a genetic definition of life. Cho *et al.* argued that further discourse in this area should be informed by perspectives from theology, philosophy, the social sciences, and the general public. They also proposed that fears of “playing God” were inconsistent with major Western religious traditions, but that scientists should take their role as stewards and the dangers of hubris seriously. It was noteworthy in the recent discussions of Gibson *et al.* that the Vatican response was fairly positive, noting that it was “important research” that had not created life, but had “replaced one of its motors” (10).

Finally, Cho *et al.* discussed the need for new models of intellectual property to ensure that both commercial and public interests were protected. Gibson *et al.* have not raised new issues regarding patenting, but current intellectual property structures remain a potential barrier to synthetic biology research and development (11).

Most commentators on the implications of synthetic genomics and synthetic biology have agreed that few, if any, new ethical issues are raised (12–19). Some, however, have reemphasized the larger questions raised about humans as creators and definers of life (13), the intrinsic value of synthetic biology independent of consequences, nonphysical harms (such as fair distribution of benefits), and the appropriate relationship between humans and the natural world (17). Molecular biologists have characterized the importance of the work by Gibson *et al.* in technological terms, whereas social scientists have portrayed it in more philosophical terms.

Although synthesis and assembly of an intact bacterial genome is a noteworthy achievement, it represents the net result of many incremental, technical advances over the past several decades and is primarily a matter of scale. Of perhaps greater technological significance is the manipulation and transplantation of an intact bacterial genome into a heterologous cell. However, the authors

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also demonstrate the ability of their synthetic construct to direct its own sustained replication, as well as the operation and replication of its new cellular “home.” This achievement lends the work a different type of importance, with largely symbolic value in the near term, but more practical and possibly strategic importance in the long term.

In general, our current ability to predict and design novel organisms with virulence (or other relevant phenotypes) *de novo* (aside from simple toxin gene cassette insertions, and so on) is fairly primitive. This paper does not address or provide new solutions to this major challenge. Instead, it presents what is

binations will be less predictable and may require different conceptual frameworks for oversight than “as safe as” regulatory standards (16), as well as creative thinking about engineered fail-safe designs and more comprehensive bioassays.

Much of the concern about synthetic biology has been focused on illegitimate users of technology. However, the ease of access to research tools and concepts increases the likelihood of unintentional effects by well-meaning institutionally based scientists or amateur biologists. Because these fields are fast-moving, complex, and accessible, ethical and policy considerations must be integrated

“To what degree and in what ways should a genome differ from previously known genomes before perceptions of security and ethical risks deserve special notice?”

largely a resynthesis of a naturally occurring genome. Nevertheless, because this work clearly lies on a trajectory leading to more substantial risk in the future and because the subject is rife with potentially misleading terms and ethically charged concepts, communication of risks and benefits and careful education of the public are critical.

The work has drawn attention to synthetic biology and its implications at the highest levels of the U.S. government (20, 21), even though there is little public awareness of the state of the science and technology, potential applications, and risks. This event provides a great opportunity for the scientific community to engage in public discourse, as well as to educate its own members about the importance of articulating the responsibilities of investigators, publishers, and industry (19).

Synthetic genomics and synthetic biology may necessitate a new model for addressing ethical and policy issues because of the complexity of the biological systems being mimicked and manipulated. The complex interactions of biological parts and their evolution will likely lead to unpredictable, emergent behavior in engineered organisms and ecosystems (16). Even addition of a single gene, e.g., encoding a well-characterized fungal toxin, to a heterologous fungal host species led to unexpected virulence and host range in infected plants (22). More complex com-

as far upstream as possible in and before the design phases of research to be effective. One question that will need to be addressed is, “To what degree and in what ways should a genome differ from previously known genomes before perceptions of security and ethical risks deserve special notice?”

Risks and benefits need to be evaluated broadly (i.e., not only in terms of safety and security but in terms of environmental, social, and economic risks and benefits) and as part of the planning of research questions and designs. Identifying and addressing an expanded notion of risk and benefit might require expertise beyond genomics, for example, in environmental or social sciences. A realistic assessment of likely benefits is important because it highlights potential issues of distributive justice and fairness, especially with growing skepticism about the practical application of genomics to date, and the tendency toward hype (23, 24). It is perhaps even more important, however, to communicate the intentions of the scientist and to minimize potential financial and other conflicts of interest. In the absence of clear communication about the rationale for synthetic genomics and synthetic biology research, the scientific community leaves itself vulnerable to growing mistrust by the lay public. Use of reductionist terms such as “programming life” or “artificial life” are questionable sci-

entifically and ethically because they vastly overstate our current ability to control biological processes at the organismal level.

By taking the lead in public discourse, scientists can maintain public legitimacy (15). During this period of scarce public resources and other competing needs, and as the motives of scientists are increasingly questioned, it behooves us to take action.

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EVOLUTION

Genes for High Altitudes

Jay F. Storz

During the past 100,000 to 200,000 years, anatomically modern humans successfully colonized a diverse range of environments across the planet. Some of the most extreme of these environments are found on the high-altitude plateaus of Central Asia and the Andes. The Tibetan Plateau appears to have been inhabited for ~25,000 years, and permanent settlements have been established at elevations of 3500 to 4500 m (1, 2). Residents of these lofty altitudes descend from a long line of highland ancestors who lived long enough to reproduce in spite of the physiological challenges associated with chronic oxygen deprivation. Thus, studies of indigenous high-altitude residents provide the opportunity to identify genes that may have played a role in hypoxia adaptation. On pages 72 and 75 of this issue, Simonson *et al.* (3) and Yi *et al.* (4), respectively, combine genomic and candidate-gene analyses to identify the genetic basis of high-altitude adaptation in Tibetans. Together with another recent analysis (5), the studies reveal that genes in the hypoxia-inducible factor (HIF) oxygen signaling pathway have been subject to strong and recent positive selection in Tibetan highlanders.

Hypoxic stress impinges on well-characterized physiological pathways related to oxidative energy metabolism, which has facilitated the identification of high-altitude adaptation mechanisms in nonhuman animals (6, 7). An alternative to this candidate-gene approach is to screen DNA sequence variations (polymorphisms) across the entire genome to identify chromosomal regions that have contributed to high-altitude adaptation. This population genomics approach holds the promise of identifying genes whose function and adaptive importance were previously unanticipated.

The HIF family of transcription factors regulates oxygen homeostasis by coordinating the transcriptional response to hypoxia. The human gene *endothelial PAS domain protein 1* (*EPAS1*, also called *HIF2 α*) encodes the oxygen-sensitive subunit of the HIF-2 transcription factor and plays an important role in regulating erythropoiesis. After conducting genome scans to identify candidate genes for high-altitude adaptation, association stud-



The high life. Tibetans, who have lived at high altitudes for nearly 25,000 years, survive the low-oxygen environment through a low blood hemoglobin concentration.

ies demonstrated that noncoding variants in and around the genes *EPAS1*, *EGLN1* (a regulator of HIF), and *PPARA* (a transcriptional target of HIF) are strongly associated with a reduced blood concentration of hemoglobin in Tibetan highlanders (3–5). Previous studies demonstrated that Tibetan highlanders are characterized by a low hemoglobin concentration relative to lowland natives who have acclimatized to high altitude (1, 8). When lowland natives ascend to high altitude, the acclimatization response to hypoxia involves an increase in red blood cell production, and hence, an elevated hemoglobin concentration. Andean residents at high altitude are also characterized by an elevated hemoglobin concentration. By contrast, Tibetans living at elevations of up to 4000 m present a hematological profile similar to what would be expected at sea level (1).

It may seem counterintuitive that a reduced hemoglobin concentration would be advantageous under conditions of chronic hypoxia at high altitude because having more hemoglobin per unit blood volume increases arterial oxygen content. However, beyond a certain threshold, an increased hemoglobin concentration results in an elevated blood viscosity (because red blood cells make up a greater fraction of the total blood volume), thereby increasing vascular resistance and compromising tissue oxygenation. At high altitude, evidence suggests that the optimal hemoglobin concentration may actually be quite close to the typical sea-level value (9). The potential fitness consequences of excessive erythropoiesis come into especially sharp focus when

Analyses of genomes from Tibetan populations reveal a signaling pathway that may account for high-altitude adaptation.

considering the effects on pregnancy outcomes at high altitude (10–12), because elevated hemoglobin concentration in the maternal circulation is associated with restricted fetal growth and increased fetal mortality in utero.

Given these adverse effects, why is an increased hemoglobin concentration such an integral part of the acclimatization response to hypoxia in humans? One possible explanation is that the HIF-mediated increase in hemoglobin concentra-

tion is a misdirected response to hypobaric hypoxia that originally evolved as a response to anemia. Hypobaric hypoxia and anemia both result in reduced tissue oxygenation, but their root causes differ. In anemia, reduced tissue oxygenation is caused by a curtailed oxygen transport capacity of the blood, and can therefore be rectified by increasing hemoglobin concentration. By contrast, in hypobaric hypoxia, the reduced tissue oxygenation has an external cause: The reduced oxygen tension of inspired air leads to a reduced oxygen saturation of arterial blood. Under these circumstances, a dramatically increased hemoglobin concentration may further impede oxygen delivery to metabolizing tissues. If our hominid ancestors were never forced to contend with the physiological challenges of low-oxygen environments, it might be unreasonable to expect that we would have evolved an appropriate physiological response to chronic hypoxia.

If certain features of the acclimatization response to hypoxia are maladaptive, then many adaptive changes in high-altitude populations may result from selection on genetically based trait variation that counteracts the effects of environmentally induced changes. In the case of hemoglobin concentration in Tibetans, for example, selection has not favored a trait value outside the ancestral range of variation. Instead, selection appears to have favored a blunted erythropoietic response such that hemoglobin concentration at high altitude is maintained at the sea-level status quo. Although the mechanism has yet to be elucidated, it appears that regulatory

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changes in *EPAS1* and other HIF-related genes have recalibrated the set point for hypoxia-induced erythropoiesis in Tibetans. Andean highlanders have not evolved a similar mechanism for attenuating the erythropoietic response to hypoxia, possibly because of their shorter history of residence at high altitude (1, 10).

It remains to be seen whether hemoglobin concentration represents the direct phenotypic target of selection in Tibetans, or whether changes in hemoglobin concentration represent an ancillary effect of selection on some other physiological trait that is altered by regulatory changes in the HIF cascade. These studies of Tibetan highland-

ers provide compelling proof of principle that the integration of population genomics and association studies can successfully identify targets of recent positive selection. These results should motivate detailed functional studies that will hopefully reveal the mechanistic basis of fitness variation among alternative genotypes at *EPAS1* and other HIF-related candidate genes for high-altitude adaptation.

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ECOLOGY

Tropical Arthropod Species, More or Less?

Robert M. May

If some alien version of the Starship Enterprise visited Earth, what might be the visitors' first question? I think it would be: "How many distinct life forms—species—does your planet have?" Embarrassingly, our best-guess answer would be in the range of 5 to 10 million eukaryotes (never mind the viruses and bacteria), but we could defend numbers exceeding 100 million, or as low as 3 million.

We could add that we began systematically naming species just a little over two centuries ago, and that our labor force is inefficiently distributed: about one-third of taxonomists work on vertebrates (at most 1% of the total number of species), one-third on plants (around 10%), and the remaining one-third on invertebrates (comprising at least 90%). Furthermore, a lack of broad, synoptic databases and problems with synonyms—the same species cataloged differently in different collections—means that we are uncertain, by around 10%, of how many distinct species we actually have named and recorded. I think the total is probably 1.6 to 1.7 million, and each year we add roughly 15,000. So even if the total number of eukaryotic species is as low as 3 million, we've a long way to go.

A recent paper by Hamilton *et al.* (1) represents a major step toward narrowing the range of estimates of the likely total. This

total is dominated by invertebrates, and more particularly, by arthropods. Hamilton *et al.*'s point of departure is an influential, though controversial, paper by Erwin (2). He began with a study of the number of beetle species found in the canopy of a single tropical tree species (*Luehea seemannii*). Then, Erwin pyramided this through a chain of argument into an estimate of 30 to 100 million tropical arthropod species. Each link in Erwin's chain is controversial, however, so I believe that his work is important for setting a defined agenda for research, rather than for its estimate as such.

It makes sense to begin an estimate of global arthropod species numbers by focusing on tropical beetles, partly because tropical arthropods dominate the global total, and partly because beetle species are both functionally diverse and represent roughly one-

New research on tropical beetles could help narrow estimates of how many species live on Earth.

third of all arthropods. The subsequent links in Erwin's chain of argument rest on a series of questions. What is the average degree of "effective specialization" (3) of herbivorous beetle species across all tree species (that is, how many different tree species does the average beetle species feed on)? What is the average fraction of all such beetle species that are herbivores? What proportion of canopy arthropod species are beetles? What fraction of all species found on the average tree species are canopy arthropods? What is the total number of tropical tree species?

Hamilton *et al.* combine a wide range of data bearing on these questions, and then use sophisticated statistical techniques and simulations to estimate answers. Essentially all the data are from studies more recent than Erwin's seminal work, and I believe most were prompted by it. Broadly, Hamilton *et al.* give two lines of analysis, model A and model B. Both models differ from Erwin's in replacing his single "educated guess" with a range of estimates accompanied by probabilities. These probability distributions are estimated from a variety of studies of tropical trees and their associated beetles and other arthropods. The data are primarily from New Guinea (which is home to roughly one-third of tropical tree species), but also from Brazil, Panama, Venezuela, Sulawesi, and other places. In particular, the research-



One of how many? Tropical arthropods, such as this bark beetle (*Dinoplatypus pallidus*) from New Guinea, are believed to make up a large fraction of all species on Earth, and have played a big role in efforts to estimate the total number of species.

ers used a large data set involving 56 New Guinea tree species (4) to estimate the number of herbivorous canopy beetle species, and their degree of effective specialization (5).

For most of the links in Erwin's chain, Hamilton *et al.*'s probability distributions do not differ much from Erwin's single values. Erwin estimates 40% of canopy arthropods to be beetles (mirroring the global fraction for all arthropods), while Hamilton *et al.* estimate an equal probability that the percentage is between 25 and 66%. Likewise, the estimated total number of tropical tree species differs only slightly: Erwin takes the total to be 50,000, whereas Hamilton *et al.* estimate from 43,000 to 50,000 tropical tree species.

The big difference, however, lies in the use of the much larger data set for the 56 New Guinea tree species. This leads, in effect, to the conclusion that the average beetle is less specialized—feeds on more different tree species—than suggested by Erwin, and thus to overall estimates for the total number of tropical arthropod species that are far lower than Erwin's 30 to 100 million.

In model A, for instance, the probability distribution suggests that the total is around 3.7 million, with a 90% probability that the number is between 2.0 and 7.4 million. Model B—essentially a refinement of model A that puts greater weight on the New Guinea tree data (6)—suggests 2.5 million tropical arthropod species, with a 90% probability that the number is between 1.1 and 5.4 million. Both models assign a very low probability (on the order of 10^{-5}) to Erwin's higher estimates.

Overall, these conclusions imply that around two-thirds of all arthropod species still await discovery and description. In part, this sorry state of affairs reflects inefficient distribution of taxonomic attention, which is disproportionately directed to the more appealing furry and feathery vertebrates. It also often reflects a tendency for funding agencies, in the United Kingdom and elsewhere, to view research on basic systematics and taxonomy as insufficiently sexy, or not conforming to simplistic Popperesque notions of falsifiable hypotheses (7). My guess is that the hypothetical aliens would take a dim view of all this.

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6. Model B acknowledges that some New Guinea tree genera are much more speciose than others. In three cases among the 56-tree data set (4), Hamilton *et al.* had more than one tree species per genus; they used a representative host and its associated beetle species (with separate randomization for each species).
7. See paragraph 5.4 from the UK House of Lords' Science and Technology Select Committee, 5th Report (2007–2008), *Systematics and Taxonomy: Follow-up* (HL Paper 162), which found that "The evidence we received about the willingness of the Research Councils to fund taxonomy was confused." The government's response said that "Research proposals including classical taxonomic approaches may have the best chance of success if they take account of (a) the hypothesis testing science that typifies responsive mode grants, ..." (chapter 5, paragraph 7.19, item 6). For further discussion see www.publications.parliament.uk/pa/ld200708/ldselect/scitech/162/162.pdf.

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CHEMISTRY

Downsizing the Hydrated Electron's Lair

Kenneth D. Jordan¹ and Mark A. Johnson²

The hydrated electron is a key reactive intermediate in many chemical reactions, including those responsible for the biological effects of radiation. When water molecules (or molecules dissolved in it) encounter radiation, they ionize and emit electrons that can react with biomolecules, often in deleterious ways. Despite being so reactive, these electrons can have appreciable lifetimes because they are stabilized by surrounding water molecules. The hydrated electron, e_{aq}^- , has been understood for more than 50 years in terms of a cavity model in which the electron is excluded from regions where the water molecules have appreciable electron density, and the void or cavity it occupies is stabilized by the interaction of the charge with the dielectric medium outside the void (1–3). On page 65 of this issue, Larsen *et al.* (4) challenge both of these tra-

ditional views with a new model in which the e_{aq}^- not only penetrates the charge distribution of the water molecules but also is associated with a region of enhanced water density rather than a cavity. The electron wave function is distributed like tentacles that wrap around and between the water molecules.

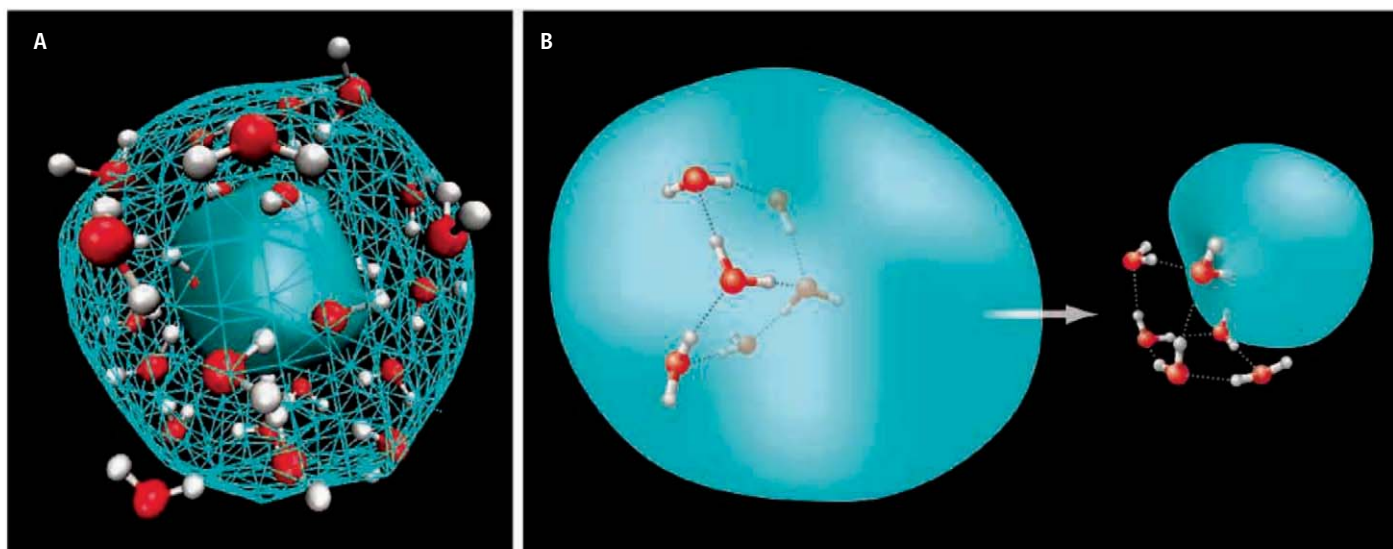
One challenge in definitively establishing the structure of e_{aq}^- is that it is observed experimentally in indirect ways. The characteristic signature of e_{aq}^- is its broad asymmetric absorption band peaking at 1.7 eV (in the red region of the visible spectrum) that is the result of e_{aq}^- forming an excited state. Analysis of this band yields a "radius of gyration" for e_{aq}^- of about 2.5 Å (5), which is generally taken to be the radius of the cavity (the volume is equivalent to about two water molecules). The first hydration shell of e_{aq}^- is most often viewed as having multiple water molecules (typically six) pointing OH groups, which bear H atoms with partial positive charge, toward the center of the cavity.

A new model for free electrons in water suggests that they are localized in regions of enhanced rather than depleted water density.

This geometry creates a sizable attractive potential that stabilizes the excess electron. Previous molecular dynamics simulations of e_{aq}^- have basically supported the cavity model. However, one persistent and troubling aspect of the cavity model is its inability to account for the incredibly fast electronic relaxation kinetics of the excited state of the excess electron. These studies use ultrafast laser pulses to create and study the excited state and have been done in liquid water as well as in model studies based on anionic water clusters (6).

The model of Larsen *et al.* can account for a wide range of properties of the hydrated electron, including the location of the absorption maximum and the excited-state dynamics, in a scenario that does not accommodate the electron in a cavity (see the figure, panel A). These new simulations use a recently developed model for the potential between an electron and a water molecule that has stronger attractive forces near the O atom relative to the potentials used in ear-

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Wet electrons. (A) The simulations by Larsen *et al.* find that the hydrated electron is in intimate contact with water molecules within the radius of gyration of the electron. Here, a representative configuration shows all water molecules within 6 Å of the center of mass of the electron. (B) Model studies on water clusters

provide additional insights into the hydrated electron in solution. Depicted here is the rearrangement of a cluster of six water molecules before and after it captures an electron. The surfaces depicting the distribution of the excess electron enclose 50% of the charge.

lier simulations of e_{aq}^- . Stronger attractions might appear to be counterintuitive because the O atom of a water molecule carries a partial negative charge, which would create a repulsive interaction for an electron at short distances. However, at very short distances (1.5 Å), where the excess electron penetrates the charge distribution of the O atom, it begins to feel an attraction from the oxygen atom nucleus.

Only a small amount of the excess electron density is calculated to fall within 1.5 Å of the O atom nucleus, but it appears to be fundamental in explaining the absence of the cavity in the simulations of Larsen *et al.* An important aspect of their contribution is that it highlights how our qualitative picture of the hydrated electron profoundly depends on subtle refinements in the electronic potentials used to describe interactions. This new model is most intriguing, but we caution that it is probably not the final word for this challenging and fascinating problem. In particular, it does not account for the states responsible for the high-energy tail observed in the electronic absorption spectrum. Such details may seem like loose ends, but an accurate description of the hydrated electron is important in other contexts. For example, e_{aq}^- serves as a paradigm for mapping complex many-body problems, where electron correlation effects are important, into simpler one-electron models.

One example of the challenges involved in accurately describing e_{aq}^- under ambient conditions is the ongoing controversy concerning the related problem of surface ver-

sus interior binding of an excess electron trapped on a cold cluster of neutral water molecules in the gas phase. At the smallest cluster sizes (two to six water molecules), the molecules form network structures that have been unambiguously established through a combination of vibrational spectroscopy and electronic structure calculations (7, 8). Even though the excess electron is generally viewed as being bound to the surfaces of these small clusters, it causes dramatic rearrangements of the supporting water networks. The rearrangement of a neutral $(H_2O)_6$ cluster after electron attachment (9) is shown in panel B of the figure.

Several research groups have extended the cluster studies to much larger sizes (up to 200 or so) (10, 11). In principle, molecular features of e_{aq}^- might be uncovered by extrapolating the cluster behavior to bulk asymptotes, but there are complications. For such an extrapolation to be meaningful, the cluster anions would have to have the excess electron localized in the interior rather than on the surface of the cluster. Although both the Neumark (10) and Issendorff (11) groups assigned the high electron binding species observed in their experiments to interior states (for clusters with 20 or more molecules), simulations from two theory groups (12, 13) have concluded otherwise. A major issue, which has been noted by these and other researchers, is that under the experimental conditions (temperatures below 150 K), the clusters are almost certainly solidlike. As such, the anions formed under typical experimental conditions are likely meta-

stable, adopting structures quite different from the hydrated electron in bulk water.

The study of Larsen *et al.* dramatically illustrates how small changes in the model potentials, at distances thought to be irrelevant, can drastically alter the structure of e_{aq}^- . Outstanding issues still remain, and a wide range of theoretical and experimental studies will be needed as we inch closer to unveiling a robust, consensus molecular description of the hydrated electron.

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A Guardian of T Cell Fate

James P. Di Santo

Within the human hematopoietic system that gives rise to all blood cell types, the generation of lymphocytes—T cells, B cells, and natural killer cells—is orchestrated at the early developmental stages, when progenitor cells selectively block alternative developmental pathways (for example, erythroid and myeloid) to become lymphoid-committed (1). On pages 89, 93, and 85 of this issue, L. Li *et al.*, Ikawa *et al.*, and P. Li *et al.*, respectively, identify the transcription factor Bcl11b as a critical determinant of this exclusion process during T cell specification (2–4).

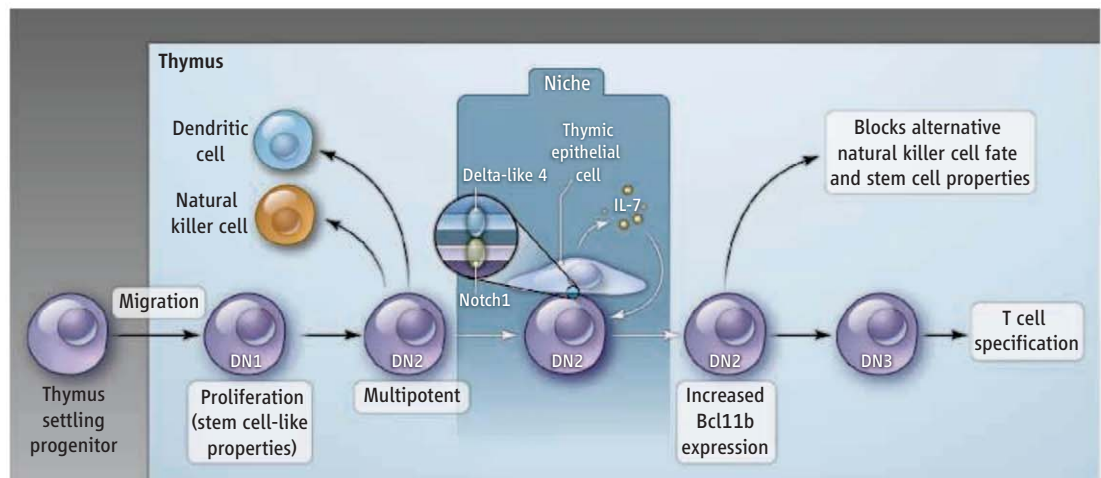
T cells arise from hematopoietic progenitor cells that migrate from the bone marrow to the thymus, where they proliferate as thymocytes. Tissue-specific signals then direct their developmental fates. The T cell specification pathway involves signaling by the Notch1 receptors expressed by early progenitors, upon interaction with cognate ligands (Delta-like proteins) expressed by thymic epithelial cells (5–7). However, Notch1 signaling alone does not guarantee completion of this process, because an early thymocyte still retains potential to become an alternate lymphoid or a myeloid cell type (8). What mechanisms, then, “lock in” commitment to the T cell fate?

The transcription factor Bcl11b is essential for specifying the T receptor (with α and β polypeptides) expressed by the majority of T cells generated in the thymus (9, 10), and in controlling thymocyte expansion (9). Li *et al.* (2) analyzed the role of Bcl11b in the early events of T cell development by culturing multipotent hematopoietic progenitor cells that had been acutely deleted of Bcl11b with stromal cells bearing the Notch ligand Delta-like 1. The absence of Bcl11b blocked progression of progenitors to a “double-

negative” stage called DN2 (referring to lack of expression of the T cell co-receptor proteins CD4 and CD8), when specification of T cells normally initiates. Although Bcl11b-deficient DN2 cells expressed normal amounts of other transcription factors associated with T cell differentiation at this stage (including *Gata3*, *Tcf7*, and *Ets1*), several genes normally suppressed at this stage were still highly expressed, including proto-oncogenes (*Tal1*, *Sfp1*, *Lyl1*, and *Erg*) and genes encod-

The repressive activity of a transcription factor allows thymocytes to shed alternative cell fates and become T cells.

(Delta-like 4) for the Notch receptor, and a cocktail of growth-promoting cytokines [interleukin-7 (IL-7), stem cell factor, and FMS-like tyrosine kinase ligand], Ikawa *et al.* generated continuous cultures of DN2 cells that retained the potential to differentiate into multiple cell types—T cell, natural killer cell, dendritic cell, and macrophage. The transcriptional profiles of developmentally arrested DN2 and DN2mt cells were similar. Ikawa *et al.* discovered that simply reducing



Becoming a T cell. Early progenitors mature through distinct stages (DN1, DN2, DN3) in the thymus. DN2 cells proliferate in response to IL-7 produced by thymic epithelial cells. Once thymocytes leave this niche, loss of IL-7 causes an increase in Bcl11b expression. This suppresses alternate lineage development, extinguishes stem cell-like gene expression, and specifies T cell fate.

ing growth factor and cytokine receptors (*Kit*, *Flt3*, and *Il7r*). Accordingly, Bcl11b-deficient progenitors could be maintained in continuous cell culture and demonstrated superior proliferation compared to wild-type cells. In addition, Li *et al.* (2) show that Bcl11b-deficient DN2 cells had increased expression of genes associated with natural killer cells (including *Id2*, *Il2rb*, and *Nfil3*). Thus, Bcl11b is a critical transcription factor that attenuates the proliferative, stem cell-like characteristics of early progenitors, and focuses T cell commitment by repressing alternative cell fates, especially the natural killer cell lineage.

Ikawa *et al.* (3) investigated extracellular signals that control lineage determination of early thymocytes. Just before full T cell commitment, thymocyte progenitor cells in the DN2 stage retain the capacity to generate myeloid cells (DN2mt cells, where mt denotes myeloid-T) (11). Using a cell culture system comprising immobilized ligand

the concentration of IL-7 in the culture system stimulated robust T cell development, indicating that IL-7 controls the expression of a critical T cell-promoting transcription factor. Bcl11b emerged as the primary candidate because Bcl11b-deficient progenitors were developmentally arrested at the DN2 stage and retained macrophage and natural killer cell potential. Expression of Bcl11b in developmentally arrested DN2 cells relieved this arrest in the presence of Delta-like 4 and IL-7. These findings identify Bcl11b as a sensor that links cytokine signaling thresholds and T cell lineage commitment in early thymocyte progenitors.

The related study by Li *et al.* (4) demonstrates that ablation of Bcl11b expression at different stages of T cell development can “reprogram” T cells into natural killer-like cells. Upon loss of Bcl11b, thymocytes that have committed to the T cell fate (the DN3 stage) lose expression of T cell lineage genes

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(such as *Ets1*, *Gata3*, and *Tcf1*) and boost the expression of natural killer cell lineage-associated genes (including *Id2*, *Il2rb*, and *Nfil3*). They also gained the capacity to grow in response to IL-2, and transcriptionally and functionally resembled normal cytokine-activated natural killer cells. As such, Li *et al.* (4) refer to these cells as induced T-to-natural killer (ITNK) cells, which could potentially eliminate tumor cells in vitro and in vivo. Remarkably, mature T cells could also be “reprogrammed” to ITNKs, although they retained some signatures of their T cell origin. Whether these ITNK cells recognize antigen via the T cell receptor is not known. Thus, Bcl11b appears critical for maintaining T cell identity throughout the T cell life span. Moreover, because of their robust proliferative capacities, ITNK could represent a potential source for therapies.

The three studies suggest a revised model of early T cell lineage commitment with Bcl11b as the focal point (see the figure). Signals through Notch1 are critical for initiating T cell specification at the early DN stage. Li *et al.* (4) show that Notch regulates Bcl11b expression, which is critical for repressing myeloid and natural killer cell poten-

tial. Nevertheless, because Bcl11b-deficient precursors do not regain B cell potential, another transcription factor must be involved in T cell specification.

Although IL-7 is essential for early thymocyte survival and proliferation (12), the results of Ikawa *et al.* (3) suggest that it must also be attenuated to allow Bcl11b-mediated T cell development. How can this paradox be resolved? It is likely that an IL-7 “niche” in the thymus is limited, as suggested by the discrete anatomical placement of IL-7-producing thymic epithelial cells (13). When IL-7 becomes limiting, DN2 cells must receive other survival signals or else die. This instance of IL-7 deprivation could increase Bcl11b expression [which in turn would repress *Il7r* expression (2)], thereby establishing a feedforward loop for subsequent T cell commitment. In this way, overgrowth of the IL-7 niche would result in default T cell progression and further maturation in an IL-7-independent manner. As a corollary, early thymocytes from IL-7-deficient mice (while severely reduced) might progress more rapidly through the DN2-DN3 transition and show unusually high Bcl11b expression.

The gene targets of Bcl11b that promote T cell development are yet to be defined. Similarly, the molecular determinants of the T cell “signature” defined by Bcl11b in mature T cells are largely unknown. How is T cell identity maintained? As IL-7 is a central mediator of peripheral lymphocyte homeostasis, it will be interesting to learn whether Bcl11b regulation of cytokine receptor sensitivity also operates in the maintenance of diverse peripheral T cell subsets.

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CHEMISTRY

To Cool or Not to Cool

Volker Bromm

One of the tantalizing questions in modern cosmology concerns how the first stars formed at the end of the cosmic dark ages, a few hundred million years after the Big Bang. It has long been realized that their emergence must have set into motion the rapid transformation of the early universe from an exceedingly simple initial state into one of ever-increasing complexity (1). On page 69 of this issue, Kreckel *et al.* (2) present a combination of laboratory and numerical astrophysics to provide an improved value for the production rate of molecular hydrogen (H_2), one of the key physical processes that governed the formation of the first stars.

A picture of primordial star formation has emerged, based on sophisticated supercomputer simulations, in which the first stars were predominantly very massive, with typical masses a few hundred times that of the Sun

(3). At the core of this picture lies the argument that the primordial gas, consisting only of the hydrogen and helium made in the Big Bang itself, can only cool via the excitation of low-energy rotational transitions in H_2 . This is not a very efficient cooling mechanism, however, resulting in temperatures of about 200 to 300 K within the pristine star-forming clouds compared to the 10 K found in present-day giant molecular clouds, where stars form in our Milky Way galaxy. In modeling the first stars, a crucial ingredient is the amount of H_2 produced. Until now, the corresponding production rate was subject to substantial disagreements between different theoretical calculations and experiments. The results of Kreckel *et al.* remove this lingering uncertainty. Indeed, it is a fascinating aspect of this study that microphysical processes can have such large-scale, cosmological implications.

The typical mass range of the first stars is so important because it determines how they shaped early cosmic evolution via feedback effects. For example, the emission of ener-

Precision measurements of chemical processes are providing a clearer picture of how the universe evolved.

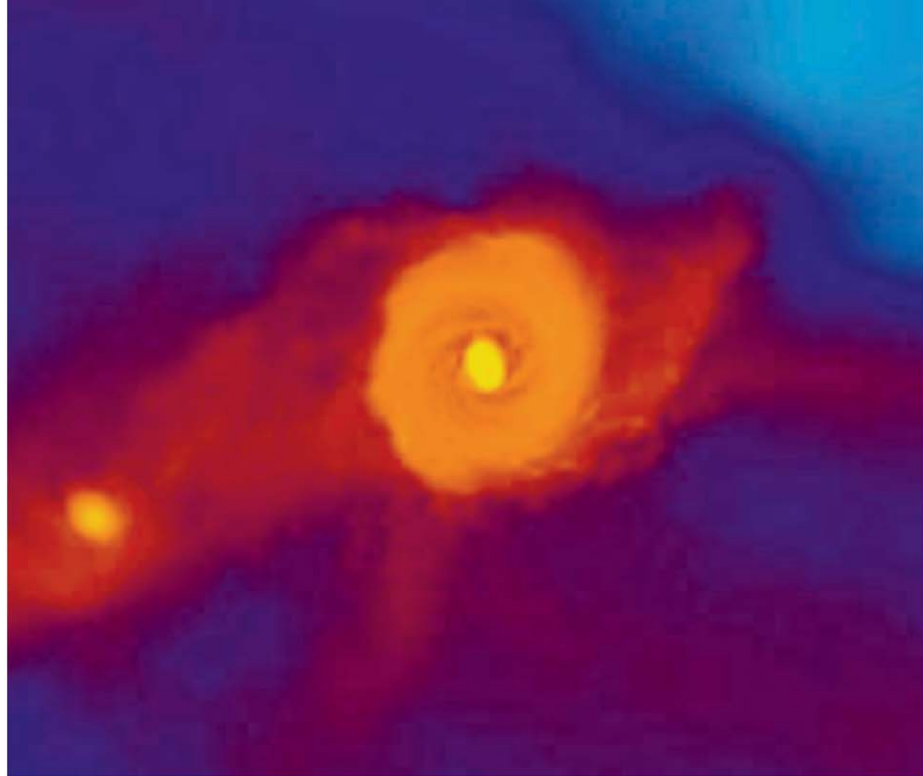
getic ultraviolet radiation from their surfaces strongly depends on stellar mass. Emitted photons were able to ionize the neutral hydrogen in the surrounding intergalactic medium, thus initiating the protracted process of cosmic “reionization.” Understanding this process is a crucial goal, as it was the last global phase transition in the history of the universe, transforming a completely neutral hydrogen-helium gas into the almost fully ionized plasma observed around us today (4). After reionization, different regions in the universe evolved largely independently of each other. Reionization left a number of observable signatures behind whose strength and character depends on the contribution from the first stars. The most important signature is the optical depth to Thomson scattering, a process that describes how often a photon of the cosmic microwave background interacts with free electrons along its path of propagation. The free electrons in turn are created by the ionizing radiation from stars, and the cumulative optical depth is thus a measure

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of star formation throughout cosmic time. The Wilkinson Microwave Anisotropy Probe (WMAP) has determined this quantity with high precision (5), indicating that a few percent of the total signal measured by WMAP may be attributable to the first stars.

The first stars were also responsible for enriching the pristine, pure H/He gas with the first heavy chemical elements. Again, the nature of this initial enrichment event is sensitive to the mass of the first stars, as differences in progenitor masses translate into different classes of supernova explosions that have particular nucleosynthetic element patterns (6). One intriguing possibility is that a fraction of primordial stars died in a particular kind of explosion, called a hyperenergetic pair-instability supernova (PISN). Such events are predicted to leave behind no compact remnant, neither a neutron star nor a black hole, and would therefore release the entire amount of heavy elements produced in their interior into the surrounding cosmic gas. A PISN would therefore constitute a very efficient mechanism to rapidly enrich the early universe with heavy elements, just a few million years after the first stars appeared (7, 8). The PISN idea existed only as a theoretical scenario (9, 10), but recently, a promising candidate for such an unusual explosion was discovered in a nearby small galaxy (11). This discovery greatly strengthens the case for such extreme explosions to have occurred in the early universe.

The process investigated by Kreckel *et al.* may be important not only for the formation of the first stars, but also of the first galaxies (see the figure). Within the current standard model of cosmological structure formation, dominated by cold (slow-moving) dark matter particles, structures are predicted to form hierarchically from the bottom up, so that smaller systems merge into ones of ever-increasing mass. The first stars are expected to emerge in small dark matter halos, as single stars or members of a small group. To form galaxies, more massive halos are needed with a correspondingly larger amount of gas for star formation, so that systems of stars can be built up. The primordial gas condensing inside the first galaxies may have been able to cool to lower temperatures, and the masses of the resulting stars would be consequently smaller. The additional cooling boost would come from the hydrogen deuteride (HD) molecule, where one deuterium atom replaces one of the hydrogen atoms in conventional H₂ (12). Whether this additional cooling mechanism will be important depends on the H₂ formation rate. Now that Kreckel *et al.* have provided a reliable value for this key process, theorists can follow the thermal history of the



Primordial galaxy. A supercomputer frame showing how the pristine hydrogen and helium gas has assembled in the center of one of the first galaxies, 500 million years after the Big Bang. The prominent disk is a few thousand light years across, with a massive gas cloud in the very center. This cloud is expected to fragment and give rise to a massive cluster of stars, possibly bright enough to be observable with the JWST. Future simulations will include the new Kreckel *et al.* results.

gas as it collapses into the first galaxies with much greater confidence. Incorporating the relevant microphysics into computer models will enable improved simulations of the assembly process of primordial galaxies, and of the star formation ensuing in them.

Making realistic predictions for the properties of the first galaxies is important in preparing for the James Webb Space Telescope (JWST) launch, planned for 2014. The JWST will have exquisite sensitivity in the near-infrared waveband, where the bulk of the radiation from very distant sources lies. Despite its unprecedented sensitivity, the JWST will not be able to detect individual primordial stars, but it would be able to image clusters containing a large number of them, and such clusters may arise inside the first galaxies. Predicting the overall energy output and the color of such primordial star clusters is highly dependent on the stellar mass, and therefore on the cooling processes that initiate the star formation process. A second reason why understanding the first galaxies matters is that they are the likely sites for the formation of the first low-mass stars. Such stars evolve sufficiently slowly that they are still around today, 13 billion years after their birth. Astronomers can hunt for local fossils of the cosmic dark ages in the halo of our Milky Way, an approach often termed “stellar archaeology” (13). The idea is to scrutinize the elemental abundance patterns observed in

their atmospheres, and to infer the properties of the first supernovae that produced these elements. The final goal is to indirectly determine the mass of the first stars.

The key to unlocking the mysteries of our cosmic beginnings relies on precision measurements carried out by atomic and molecular physicists in laboratories here on Earth. The increased power of our computer models and the enhanced sensitivity of our telescopes will continue to feed the need for such precision experiments.

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The Unconscious Will: How the Pursuit of Goals Operates Outside of Conscious Awareness

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People often act in order to realize desired outcomes, or goals. Although behavioral science recognizes that people can skillfully pursue goals without consciously attending to their behavior once these goals are set, conscious will is considered to be the starting point of goal pursuit. Indeed, when we decide to work hard on a task, it feels as if that conscious decision is the first and foremost cause of our behavior. That is, we are likely to say, if asked, that the decision to act produced the actions themselves. Recent discoveries, however, challenge this causal status of conscious will. They demonstrate that under some conditions, actions are initiated even though we are unconscious of the goals to be attained or their motivating effect on our behavior. Here we analyze how goal pursuit can possibly operate unconsciously.

As humans, we generally have the feeling that we decide what we want and what we do. These self-reflections remind us that we are not bound to the present environment for our actions: We can envision ourselves in different places, in alternative futures, doing different things. We only have to decide to do so, and we can go see a movie tonight or hang out with friends in a bar. It is up to us. Our behaviors seem to originate in our conscious decisions to pursue desired outcomes, or goals.

Scientific research, though, suggests otherwise. In a remarkable experiment conducted more than 25 years ago (1), research participants were instructed to freely choose when to move their index fingers while the timing of the action itself, of its preparation in the brain, and of when the person became aware of the decision to act were measured. Although the decision did indeed precede the action, the preparation of the finger movement in the brain was well on its way by the time people consciously decided to act. Apparently, when people are persuaded to consciously set a goal to engage in behavior, their conscious will to act starts out unconsciously.

The finding that the pursuit of the goals that we consciously set and adopt is prepared unconsciously, at least in the earliest moments before we act on them, is intriguing. Recent research in social cognition, however, goes even one step further. This research shows that goals themselves can arise and operate unconsciously. Social situations and stimuli in the surroundings activate or prime goals in people's minds outside of their awareness, thereby motivating and guiding them, for example, to work harder on a task (2), to reach out a helping hand to others even when facing obstacles (3), or to ensure



Fig. 1. The painting *Achilles Slays Hector* by Peter Paul Rubens depicts a scene from the *Iliad* in which no reference whatsoever is made to conscious decisions or intentions (52). Instead, the pursuits of Achilles and the other characters are determined by external factors, such as fate or the gods. Here we argue that although people may have the feeling that their behavior is the result of their conscious decisions, their goal pursuits too are often directed by external sources of which they are not conscious.

that they can socialize and hang out with friends (4). Thus, goals and their pursuit can be influenced by unconscious sources, and these goals do not need to be consciously set and adopted before their influence begins to operate (Fig. 1).

A Brief Chronology

The notion that the pursuit of goals can occur unconsciously is reminiscent of Sigmund Freud,

who proposed that our (often sexual) desires are suppressed and banished to the dark corners of the mind but pop up in hysteria and under hypnosis. Whereas Freud's complex theory on the unconscious was largely unfalsifiable (5), researchers in the behaviorist tradition built more-testable theories, according to which neither consciousness nor cognition but rigid responses to environmental stimuli determine behavior.

Obviously, the environment plays a crucial role in directing behavior. However, acting on fixed stimulus-response rules—such as smashing a beeping alarm clock in the morning—is not the whole story. A substantial part of human behavior can only be explained by assuming that people have goals in mind that direct their behavior in a dynamic world (6). Cognitive scientists indeed proposed that the flexibility to produce the same desired outcomes under varying circumstances comes from our capacity to mentally represent what we want and do: to build and store mental representations of goals. These goal representations function as beacons for behavior, motivating action and guiding its course (7).

For a long time, it was generally assumed that many of the mental processes that make goal pursuit possible require consciousness. But in the past decade or so, the scientific study of goal pursuit has discovered that these processes can also operate without conscious awareness, and hence, human behavior may originate in a kind of unconscious will. This recent evidence that goal pursuit can occur without people being conscious of the active goal or its influence on their motivation and behavior has been met with resistance and skepticism, perhaps partly due to its far-reaching implications for our understanding of consciousness and for our view of what it is to be human (8). Furthermore, scientists have not come to grips with the potential redundancy of consciousness in (seemingly) volitional behavior, because the mechanism by which the activation of goal representations can produce goal pursuit unconsciously is not fully understood. Understanding this mechanism is especially important

because unconscious goal pursuit is proposed to play a key role in many aspects of social life, such as consumer and health behavior, moral behavior, and social discrimination (9).

Here we review research demonstrating that goals and the motivation to pursue them can arise unconsciously, and we propose a mechanism for how this may happen. This proposed mechanism is based on the idea that, in principle, the mind

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(and the brain in which it resides) is designed for action, and continuously and largely unconsciously processes behavioral-relevant information to readily “tell” its owner what she wants and should do to deal with the opportunities and challenges presented by the environment. Thus, setting, pursuing, and realizing goals can occur without conscious interventions.

Evidence for Unconscious Goal Pursuit

Unconscious goal pursuit was first systematically examined in social psychological experiments that made use of so-called “unrelated studies” setups. Bargh and colleagues (2) used such a setup to prime the goal to achieve—a desired outcome most people strive to attain—in U.S. students without them becoming aware of being influenced. Students were seated at a table to work on two seemingly unrelated language puzzles. For some students, the first puzzle included words related to achievement (such as win or achieve), and for others it did not. Students who were exposed to achievement words were found to outperform the others on the second puzzle. Furthermore, achievement priming was found to prompt behavioral qualities that are characteristic of motivational states or volition, such as persistence in solving puzzles and increased flexibility on the Wisconsin Card Sorting Task (10), a standard measure of flexibility in cognitive processing (11). Extensive debriefing revealed that the students did not perceive an influence of the first task (in which they were exposed to consciously visible achievement-related words) on their responses to the second. Hence, the effect of achievement priming on subsequent performance and cognitive flexibility was likely to be the result of unconscious processes.

Several experiments using the unrelated studies setup have replicated these goal-priming effects with different goals and different primes. It has been found that reading words related to cooperation causes people to work together in economic games (2) and that perceiving words describing occupations associated with making money (such as stockbroker) or inferring this goal from another person’s actions (such as a person operating a slot machine) makes them work harder when money is at stake (12). Furthermore, it has been shown that people’s pursuits are influenced by subtle cues in the environment outside their awareness: Upon entering an office, people become more competitive when seeing a leather briefcase placed on the desk (13), talk more softly when looking at a library picture on the wall (14), and clean their table more when there is a vague scent of cleaning agent in the air (15). Together, these results show that goal pursuit is influenced and controlled unconsciously by social features that have become associated with goals, either through direct practice or through social norms, communication with important others, or the media.

The studies on unconscious goal pursuit alluded to above, however, are sometimes

criticized for allowing participants to be aware of the primes. Even though participants report being unaware of the influence of the goal priming on their behavior, they still could have formed conscious intentions at the moment when they consciously perceived the goal information. Hence, their goal pursuit may still have been caused by their conscious will.

To offer even more compelling evidence for unconscious goal pursuit, researchers have recently resorted to more stringent methods such as subliminal stimulation, which prevents conscious perception of the primes. Subliminal stimulation refers to the presentation of stimuli with an intensity that is too low to reach the threshold of conscious awareness. Typically, people cannot consciously detect these stimuli, but they are nevertheless influenced by them. Whether subliminal stimulation can convey

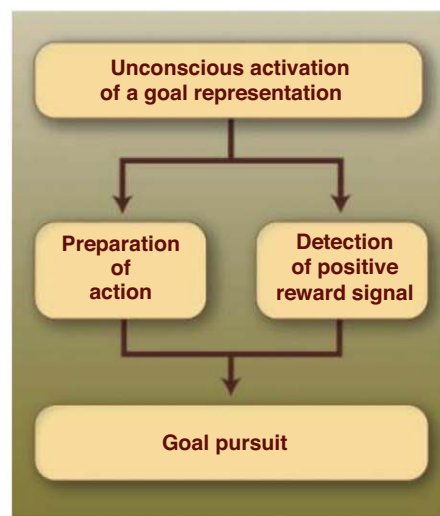


Fig. 2. The proposed mechanism for unconscious goal pursuit.

meaningful information has been debated for quite some time (16). However, recent findings provide compelling evidence that subliminal primes affect people’s responses (17), activate semantically related knowledge (18), and even influence cognitive control in tasks (19). Building on these findings, research has demonstrated effects of subliminal stimulation on goal pursuit, such as increased task performance after priming of achievement-related words (20), enhanced fluid consumption in a taste task after priming of drinking-related words (21), and an increase in instrumental behavior leading to specific goals (such as helping another person by providing useful comments) after priming of names of significant others (such as a good friend) or occupations (such as nurse) associated with these goals (12, 22).

It is important to note that in most studies on subliminal goal priming, people are asked in retrospect to indicate whether they were moti-

vated to pursue the primed goal. The general finding of these checks is that although people’s reported motivation often correlates with their behavior (people who worked harder reported that they were more motivated), these reports are not influenced by the primes. This suggests that the reason that subliminal priming of the goal affects goal pursuit is not that people become conscious of their motivation to pursue the goal after it is primed. Participants may become conscious of their motivation after the behavior is performed and when they are explicitly asked to reflect on it. In other words, the reported conscious experience of pursuing goals may be an inference rather than the cause of goal pursuit (23, 24).

In sum, a large body of research indicates that the pursuit of goals can be evoked outside of awareness. People become motivated to initiate and exhibit behaviors available in their repertoire when goals that are represented as desired outcomes are primed, even though they are not aware of the primed goal or its effect on their motivation and behavior. But how can this happen?

A Mechanism for Unconscious Goal Pursuit

To understand how the pursuit of goals occurs unconsciously, it is important to examine the functions that make motivated, goal-directed behavior possible. Fortunately, behavioral scientists have worked hard to reduce the processes involved in goal pursuit to a few basic principles. Whereas these principles have been modeled in different psychological terms, most models of goal pursuit share the three following basic features: a person (i) takes a possible outcome or goal in mind; (ii) considers whether the actions and resources to attain the outcome are available; and (iii) assesses the value of the outcome (that is, the extent to which it is rewarding or desirable) (25). Thus, whether people set an outcome that comes to mind as a goal to pursue depends on its attainability and desirability.

The assessment of this attainability and desirability of a goal is considered to require consciousness by most dominant theories on goal pursuit. Yet the evidence for unconscious goal pursuit suggests that this does not have to be the case. The notion that goal pursuit can operate unconsciously sounds ridiculous to some people but sensible to others. Sensible or not, the increasing evidence on this issue is fairly silent about the mechanisms that allow people to pursue goals without conscious awareness. We will now discuss a mechanism for unconscious goal pursuit and demonstrate that people can unconsciously detect the reward value of a primed goal and prepare feasible actions that make the goal attainable. We will then show how these two processes work together to produce goal pursuit outside people’s conscious awareness (Fig. 2).

Unconscious action preparation and execution. People may often become conscious of the actions

they prepare and execute, but their conscious knowledge of what exactly they do to reach a goal is surprisingly limited (26). Consider the muscle contractions and relaxations that make our arm grab a cup of coffee. Computational models have been proposed that describe how motoric processes and sensory feedback work together to control this behavior (27), but on a conscious level we have no idea how we do it. We just think of grabbing the cup and it happens.

We are able to initiate actions by thinking about their outcomes, because actions and their outcomes are associated on a perceptual, sensory, and motor level (28). Through prior learning, certain patterns of muscle contraction and relaxation have become associated with their observable outcomes (such as grabbing and lifting a cup). Because of these associations, bringing to mind the representation of an outcome prepares and controls perception and action to produce the outcome without much thought (6, 29). This way, action follows from an ideomotor principle (30): The mere activation of the idea of a behavioral act or outcome moves and programs the human body without a conscious decision to act. Research on social cognition and neuroscience has indeed revealed that merely seeing or reading about a behavioral act or outcome immediately increases the tendency to realize it, even when this “idea” is triggered outside of conscious awareness (31, 32).

The ideomotor principle does not hold only for the preparation and execution of simple goal-directed responses such as grabbing a cup, but also for cognitive control and more complex social behavior. For instance, in a recent experiment (19), participants had a goal to judge words in terms of either sound or meaning, depending on the visual cue preceding the word. Sometimes, the cue that corresponded to the opposite task was subliminally presented before the actual cue. It was found that the subliminally primed cue enhanced brain activity in the cortical areas related to the corresponding goal (involved in either auditory or semantic processing), whereas activity related to the consciously cued goal was reduced. The cognitive control system, in other words, responded to the subliminal cues by selecting and preparing the execution of the corresponding goal.

Research on how people perform more complex behavior has also demonstrated that when they frequently select a goal (such as going to work), they are not only able to orchestrate and execute actions that are instrumental in realizing it without conscious attention to their behavior (absentmindedly driving one's car to the office). Priming the goal immediately selects the actions themselves (33). As such, the attainment of many goals is a straightforward affair: People automatically select and execute behaviors available in their repertoire when a goal is primed, and unconsciously adjust their behavior based on perceptual input in the current situation to reach it (26, 34).

Unconscious reward processing and motivation. The pursuit of goals does not only involve

the preparation and execution of the proper behavior upon the priming of a goal representation. Because the world is a dynamic place that is full of opportunities and distractions, people should be both flexible (performing actions in new settings or switching from one goal to the other) and persistent (keeping one's eye on the selected goal) to optimize goal pursuit. People therefore also take into account the value or rewarding properties of the goal, because this tells them whether it is warranted to invest the effort or recruit the resources necessary for maintaining their behavior, overcoming obstacles, or deviating from routines to attain the goal. Hence, even though priming a goal prepares and programs actions unconsciously, whether this goal will flexibly control information processing and behavior depends on whether it is worth pursuing. This crucial step from preparing actions to actually pursuing a goal is assumed to require an act of conscious will (35, 36). So, can people determine whether it is worth pursuing a given goal and invest effort in attaining it without the involvement of consciousness? Recent studies on the basic role of the processing of reward cues in human motivation suggest that yes, they can.

Neuroimaging research has discovered that reward cues are processed by limbic structures such as the nucleus accumbens and the ventral striatum. These subcortical areas play a central role in determining the rewarding value of outcomes and are connected to frontal areas in the cortex that facilitate goal pursuit (37). These reward centers in the brain respond to evolutionarily relevant rewards such as food and sexual stimuli, but also to learned rewards (such as money or status), or words (such as good or nice) that are associated with praise or rewards (38). This demonstrates that regardless of their shape or form, such positive stimuli induce a reward signal that is readily picked up by the brain (39).

Other recent research has demonstrated that subliminal primes that are specifically related to rewards can motivate people to increase the effort they invest in behaviors. In one study (37), participants could earn money by squeezing a handgrip. Before each squeeze, the money that could be earned was indicated by a 1-pound or 1-penny coin on the screen. Whereas on some trials the coin was clearly visible, on others it was presented subliminally. Thus, effects of conscious and unconscious reward cues could be compared within one experiment. It was found that people squeezed harder on high than on low reward trials, regardless of whether the reward was consciously visible or not. Moreover, this effect was accompanied by activation in the brain areas that play a role in reward processing and the recruitment of effort for action. Similar effects of unconscious (and conscious) monetary rewards have been shown in cognitive tasks that require flexibility and cognitive resources (40, 41). These findings indicate that conscious and unconscious reward cues have similar effects on effort and flexible cognitive processing, which suggests that

conscious awareness of rewards is not needed for goal pursuit to occur.

The observation that a variety of reward cues are encoded by the same brain system to motivate cognition and action and can be processed unconsciously has led to the proposal that a positive reward signal associated with outcomes plays a crucial role in unconscious goal pursuit (42). Specifically, when a desired outcome or goal is primed, activation of the mental representation of this outcome is immediately followed by the activation of an associated positive affective tag, which acts as a reward signal for pursuing the primed goal. The positive reward signal attached to a goal thus unconsciously facilitates the actual selection of the goal and the subsequent mobilization of effort and resources to maintain the goal, unless other (more rewarding) goals gain priority (43, 44). This affective-motivational process relies on associations between the representations of outcomes and positive reward signals that are shaped by one's history (for example, when a person was happy when making money or performing well). In this case, the goal is said to preexist as a desired state in the mind. Priming this goal representation not only prepares the appropriate instrumental actions but also motivates behavior, rendering it persistent and flexible, directed at attaining the desired outcome.

We investigated the role of this positive reward signal in the effect of subliminal goal-priming in teenagers and young adults (45). They were seated in front of a computer, allegedly to test their computer mouse skills. Before starting on this test, some participants were subliminally exposed to words related to the goal of socializing on the computer screen, whereas others were exposed to words unrelated to this goal. At the onset of the mouse-skill test, they were told that if there would be enough time left after the test, they could engage in a lottery in which they could win tickets to a popular student party. Thus, spending more effort (by working faster) on the mouse-skill test was instrumental in attaining the goal to socialize. The participants indeed worked harder on the mouse-skill test when the socializing goal was primed, and this effect was stronger when socializing evoked a stronger positive reward signal in the minds of the participants (which was assessed in a separate implicit affective association task). Importantly, checks indicated that priming caused participants to pursue the goal independently of their reported motivation to attain it. This finding not only demonstrates that people invest effort as a result of subliminal goal priming but also that the resulting behavior is flexible, because people pursued an action that was available in their repertoire (skillfully using a computer mouse) but was novel to attain the goal. Similar effects of reward value have been documented for other, perhaps more consequential, behaviors. Priming an egalitarian goal, for instance, changes people's voting behavior to the extent that this goal is represented as positive or rewarding (46).

The findings discussed above indicate that the pursuit of goals occurs “out of the blue” when a goal representation is primed and followed by the activation of a positive reward signal due to an established association. However, a preexisting association between a goal representation and a reward signal may not be the only source from which unconscious goal pursuit arises. Under some circumstances, unconscious goal pursuit emerges when goal representations are primed together with positive reward signals. This ability to respond to the mere coactivation of goal representations and positive affective cues is thought to play a fundamental role in social learning (47) and is considered as basic in motivational analyses of human behavior (39). Thus, when a child observes his mother smile when munching homemade cookies, a student hears a hilarious joke upon entering the classroom, or a person strolling around a mall hears people laugh while reading on a billboard “start your holiday here,” this can cause the goal representations that are primed by those situations (eating cookies, achieving at school, booking a vacation) to acquire an intrinsic reward value, which prepares and motivates goal-directed behavior.

A recent study examined the effects of coactivating goal representations and positive reward signals on the preparation and motivation of behavior in more detail. In this study, healthy young adults had to squeeze a handgrip in response to a start sign while the timing and persistence of their behavior were measured (48). Before this task, words pertaining to the goal of physical exertion were subliminally presented or not, together with positive words that signal rewards (such as good or nice) or not. In line with the ideomotor principle, participants who were subliminally primed with the goal of exertion started to squeeze earlier. However, only participants for whom the goal was coactivated with a positive reward signal recruited more resources to execute this goal, as was evidenced by more forceful and persistent squeezing. Again, consciously reported motivation did not show any relation to the subliminal goal priming manipulation. Hence, activating a goal representation gives behavior a head start, whereas the accompanying reward signal motivates behavior outside awareness. Other studies have shown that this coactivation procedure yields effects that are similar to those of conscious goals (induced by conscious goal instructions or by making people aware of their current needs) in tasks that require flexibility and effort in novel situations (42, 49).

Conclusion

The present review and analysis reveal that the basic processes necessary for goal pursuit—preparing and directing instrumental actions and assessing the reward value of the goal—can operate outside conscious awareness. Although it is often taken for granted that goal pursuit originates in conscious decisions, it can also arise from unconscious sources. This remarkable capac-

ity for unconscious goal pursuit results from the design and workings of the brain and mind, which process and represent behaviorally relevant information in such a way that goal pursuit can be controlled by the social situation without conscious awareness of the activation and operation of the goal.

Earlier research has shown that action goals, such as moving a finger, that were initially consciously set are unconsciously prepared before they are acted on (1). The literature reviewed here suggests that the unconscious nature of the will has an even more pervasive impact on our life. Goals far more complex than finger movements, can guide behavior without being consciously set first, when they themselves are activated outside conscious awareness. These unconsciously activated goals cause people to invest effort and select actions available in their repertoire to attain the goal in novel settings without them being aware of the goal or its operation. Overall, the evidence on unconscious goal pursuit indicates that the control of unconscious goals is flexible and effortful, suited to meet the dynamics of the environment.

Understanding exactly how unconscious goals flexibly control behavior remains a challenge for future research. It has been argued that goals direct attention and behavior, even in the absence of conscious awareness of the goal (44, 50). That is, the operation of higher cognitive processes supporting goal pursuit (also conceptualized as working memory or executive control) does not care much about the conscious state of the individual. This view concurs with recent insights that attention and consciousness are distinct (51).

The research discussed here suggests that conscious goals (often induced by explicit task instructions) and unconscious goals (induced by priming) have similar effects on tasks that rely on executive control. However, it is too early to conclude that consciousness is redundant in the pursuit of goals, as we do not yet know whether there are special cases in which consciousness (apart from attention) facilitates performance. In fact, we only know that we can become consciously aware of the decisions that we make and the goals we pursue without having a proper empirical test telling us how consciousness itself exactly influences our behavior. Future research will have to explore when consciously and unconsciously activated goals direct attention and information processing in similar or distinct manners to recruit the cognitive functions and brain systems that translate goals into behavior.

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Graphite in an Apollo 17 Impact Melt Breccia

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Although condensed organic phases coating lunar fines have been previously reported (1), studies of carbon in lunar rocks could not identify discrete carbon phases, except for carbides and solar wind implanted carbon [e.g., (2)]. Here, we report on the detection of discrete multiple micrometer-sized graphite phases within an Apollo 17 impact breccia.

This sample (72255) was collected from landslide material at Taurus-Littrow. It is an aphanitic impact-melt breccia (3, 4) with a dark, fine-grained equigranular crystalline matrix containing larger clasts. The youngest material contained in the sample is dated to $\sim 3.84 \times 10^9$ years ago, which is the age assigned to the Serenitatis impact basin.

We conducted two- and three-dimensional confocal Raman imaging spectroscopy (CRIS) on a thin section and on fresh fracture surfaces of sample 72255 (5) (fig. S1). Figure 1 shows a dark area of aphanitic matrix (DAAM) with a border to the surrounding lighter material (Fig. 1A). There is a greater concentration of 2- to 8- μm dark blebs within the darker area (Fig. 1B). Graphite spectra from 72255 exhibit a range of G to D band ratios (~ 1585 and $\sim 1345\text{ cm}^{-1}$, respectively) (Fig. 1C); however, the G band peak center positions and full width at half maximum (FWHM) measurements confirm that these phases are all crystalline graphite. The ratio of the 2D ($\sim 2690\text{ cm}^{-1}$) to D band peaks increases between the three spectra (from I to III), showing the presence of graphite and rolled graphene sheets and graphite whiskers (GWs). The Raman spectrum of a GW is unique among carbon

allotropes, and it is identified by an unusually intense 2D overtone mode ($\sim 2690\text{ cm}^{-1}$) (6, 7).

In all, we imaged an $\sim 0.5\text{-mm}^2$ area of thin section 72255,89, with over 68 occurrences of subsurface (2 to 8 μm within the surface) graphite blebs between ~ 2 to 6 μm in diameter (i.e., Fig. 1, D and E). Seven occurrences of subsurface GWs, one of which was mapped in three dimensions, occur between 3 and 8 μm within the section (Fig. 1I). All instances of graphitic carbon were restricted to the 0.1-mm^2 area analyzed around the DAAM pocket (I). The majority of blackened blebs in this area are ilmenite. Most occurrences of graphite appear to be within fused grain boundaries in the matrix (Fig. 1H), although some appear to occur within single discrete grains (Fig. 1H, white and yellow areas). No graphite phases are found in the 0.4-mm^2 area of lighter material that we have analyzed, although common carbon contamination phases occur all across the thin section (fig. S2).

Several lines of reasoning confirm that the observed graphite and GWs are indigenous to the sample (5). In particular, all known GW synthesis methods involve deposition from a carbon-containing gas at relatively high temperatures ranging from 1273 to $\sim 3900\text{ K}$ (6, 7). Thus, the GWs identified in 72255 cannot have been synthesized as a result of sample handling and preparation. Moreover, they could not have been implanted by solar wind, because this carbon is typically too small to identify structurally at the magnifications used (1, 2). The crystalline graphite grains detected here are likely either intact remnants of graphite and GWs from the

Serenitatis impactor, or they could have formed from condensation of carbon-rich gas released during impact. This study indicates that impact may be another process by which GWs can form in our solar system. Furthermore, it appears carbonaceous material from impacts at the time of the Late Heavy Bombardment (LHB), and at a time when life may have been emerging on Earth, does survive on the Moon.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/329/5987/51/DC1

Materials and Methods

Fig. S1

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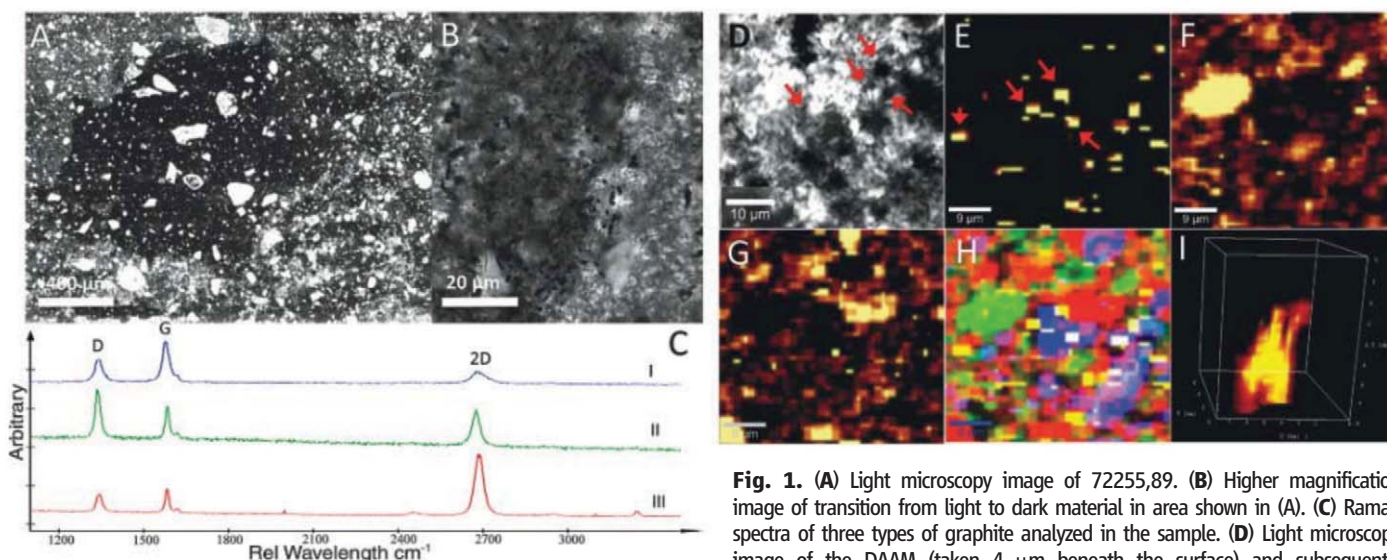


Fig. 1. (A) Light microscopy image of 72255,89. (B) Higher magnification image of transition from light to dark material in area shown in (A). (C) Raman spectra of three types of graphite analyzed in the sample. (D) Light microscope image of the DAAM (taken 4 μm beneath the surface) and subsequently analyzed by CRIS. (E to H) CRIS maps of (E) graphite 2D ($\sim 2650\text{ cm}^{-1}$) band map. Red arrows correspond to dark blebs indicated by similar arrows in (D). (Color scale is thermal; lighter areas represent higher signal intensity.) (F) Feldspar, (G) pyroxene, and (H) four-color map with green indicating feldspar; red, pyroxene; blue, olivine; and yellow, graphite. (I) Three-dimensional depth profile of the graphite 2D band from the surface to 8 μm beneath the surface of the sample (2- μm ticks).

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Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

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We report the design, synthesis, and assembly of the 1.08-mega-base pair *Mycoplasma mycoides* JCVI-syn1.0 genome starting from digitized genome sequence information and its transplantation into a *M. capricolum* recipient cell to create new *M. mycoides* cells that are controlled only by the synthetic chromosome. The only DNA in the cells is the designed synthetic DNA sequence, including “watermark” sequences and other designed gene deletions and polymorphisms, and mutations acquired during the building process. The new cells have expected phenotypic properties and are capable of continuous self-replication.

In 1977, Sanger and colleagues determined the complete genetic sequence of phage ϕ X174 (1), the first DNA genome to be completely sequenced. Eighteen years later, in 1995, our team was able to read the first complete genetic sequence of a self-replicating bacterium, *Haemophilus influenzae* (2). Reading the genetic sequence of a wide range of species has increased exponentially from these early studies. The ability to rapidly digitize genomic information has increased by more than eight orders of magnitude over the past 25 years (3). Efforts to understand all this new genomic information have spawned numerous new computational and experimental paradigms, yet our genomic knowledge remains very limited. No single cellular system has all of its genes understood in terms of their biological roles. Even in simple bacterial cells, do the chromosomes contain the entire genetic repertoire? If so, can a complete genetic system be reproduced by chemical synthesis starting with only the digitized DNA sequence contained in a computer?

Our interest in synthesis of large DNA molecules and chromosomes grew out of our efforts over the past 15 years to build a minimal cell that contains only essential genes. This work was inaugurated in 1995 when we sequenced the genome of *Mycoplasma genitalium*, a bacterium with the smallest complement of genes of any known organism capable of independent growth in the laboratory. More than 100 of the 485 protein-coding genes of *M. genitalium* are dispensable when disrupted one at a time (4–6).

We developed a strategy for assembling viral-sized pieces to produce large DNA molecules that enabled us to assemble a synthetic *M. genitalium* genome in four stages from chemically synthesized DNA cassettes averaging about 6 kb in size. This was accomplished through a combination of in vitro enzymatic methods and in vivo recombination in *Saccharomyces cerevisiae*. The whole synthetic genome [582,970 base pairs (bp)] was stably grown as a yeast centromeric plasmid (YCp) (7).

Several hurdles were overcome in transplanting and expressing a chemically synthesized chromosome in a recipient cell. We needed to improve methods for extracting intact chromosomes from yeast. We also needed to learn how to transplant these genomes into a recipient bacterial cell to establish a cell controlled only by a synthetic genome. Because *M. genitalium* has an extremely slow growth rate, we turned to two faster-growing mycoplasma species, *M. mycoides* subspecies *capri* (GM12) as donor, and *M. capricolum* subspecies *capricolum* (CK) as recipient.

To establish conditions and procedures for transplanting the synthetic genome out of yeast, we developed methods for cloning entire bacterial chromosomes as centromeric plasmids in yeast, including a native *M. mycoides* genome (8, 9). However, initial attempts to extract the *M. mycoides* genome from yeast and transplant it into *M. capricolum* failed. We discovered that the donor and recipient mycoplasmas share a common restriction system. The donor genome was methylated in the native *M. mycoides* cells and was therefore protected against restriction during the transplantation from a native donor cell (10). However, the bacterial genomes grown in yeast are unmethylated and so are not protected from the single restriction system of the recipient cell. We overcame this restriction barrier by methylating the donor DNA with purified methylases or

crude *M. mycoides* or *M. capricolum* extracts, or by simply disrupting the recipient cell’s restriction system (8).

We now have combined all of our previously established procedures and report the synthesis, assembly, cloning, and successful transplantation of the 1.08-Mbp *M. mycoides* JCVI-syn1.0 genome, to create a new cell controlled by this synthetic genome.

Synthetic genome design. Design of the *M. mycoides* JCVI-syn1.0 genome was based on the highly accurate finished genome sequences of two laboratory strains of *M. mycoides* subspecies *capri* GM12 (8, 9, 11). One was the genome donor used by Lartigue *et al.* [GenBank accession CP001621] (10). The other was a strain created by transplantation of a genome that had been cloned and engineered in yeast, YCpMmyc1.1- Δ typeIIIres [GenBank accession CP001668] (8). This project was critically dependent on the accuracy of these sequences. Although we believe that both finished *M. mycoides* genome sequences are reliable, there are 95 sites at which they differ. We began to design the synthetic genome before both sequences were finished. Consequently, most of the cassettes were designed and synthesized based on the CP001621 sequence (11). When it was finished, we chose the sequence of the genome successfully transplanted from yeast (CP001668) as our design reference (except that we kept the intact typeIIIres gene). All differences that appeared biologically significant between CP001668 and previously synthesized cassettes were corrected to match it exactly (11). Sequence differences between our synthetic cassettes and CP001668 that occurred at 19 sites appeared harmless and so were not corrected. These provide 19 polymorphic differences between our synthetic genome (JCVI-syn1.0) and the natural (nonsynthetic) genome (YCpMmyc1.1) that we have cloned in yeast and use as a standard for genome transplantation from yeast (8). To further differentiate between the synthetic genome and the natural one, we designed four watermark sequences (fig. S1) to replace one or more cassettes in regions experimentally demonstrated [watermarks 1 (1246 bp) and 2 (1081 bp)] or predicted [watermarks 3 (1109 bp) and 4 (1222 bp)] to not interfere with cell viability. These watermark sequences encode unique identifiers while limiting their translation into peptides. Table S1 lists the differences between the synthetic genome and this natural standard. Figure S2 shows a map of the *M. mycoides* JCVI-syn1.0 genome. Cassette and assembly intermediate boundaries, watermarks, deletions, insertions, and genes of the *M. mycoides* JCVI syn1.0 are shown in fig. S2, and the sequence of the transplanted mycoplasma clone sMmYCp235-1 has been submitted to GenBank (accession CP002027).

Synthetic genome assembly strategy. The designed cassettes were generally 1080 bp with 80-bp overlaps to adjacent cassettes (11). They were all produced by assembly of chemically

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synthesized oligonucleotides by Blue Heron (Bothell, Washington). Each cassette was individually synthesized and sequence-verified by the manufacturer. To aid in the building process, DNA cassettes and assembly intermediates were designed to contain Not I restriction sites at their termini and recombined in the presence of vector elements to allow for growth and selection in yeast (7, 11). A hierarchical strategy was designed to assemble the genome in three stages by transformation and homologous recombination in yeast from 1078 1-kb cassettes (Fig. 1) (12, 13).

Assembly of 10-kb synthetic intermediates. In the first stage, cassettes and a vector were recombined in yeast and transferred to *Escherichia*

coli (11). Plasmid DNA was then isolated from individual *E. coli* clones and digested to screen for cells containing a vector with an assembled 10-kb insert. One successful 10-kb assembly is represented (Fig. 2A). In general, at least one 10-kb assembled fragment could be obtained by screening 10 yeast clones. However, the rate of success varied from 10 to 100%. All of the first-stage intermediates were sequenced. Nineteen out of 111 assemblies contained errors. Alternate clones were selected, sequence-verified, and moved on to the next assembly stage (11).

Assembly of 100-kb synthetic intermediates. The pooled 10-kb assemblies and their respective cloning vectors were transformed into yeast as

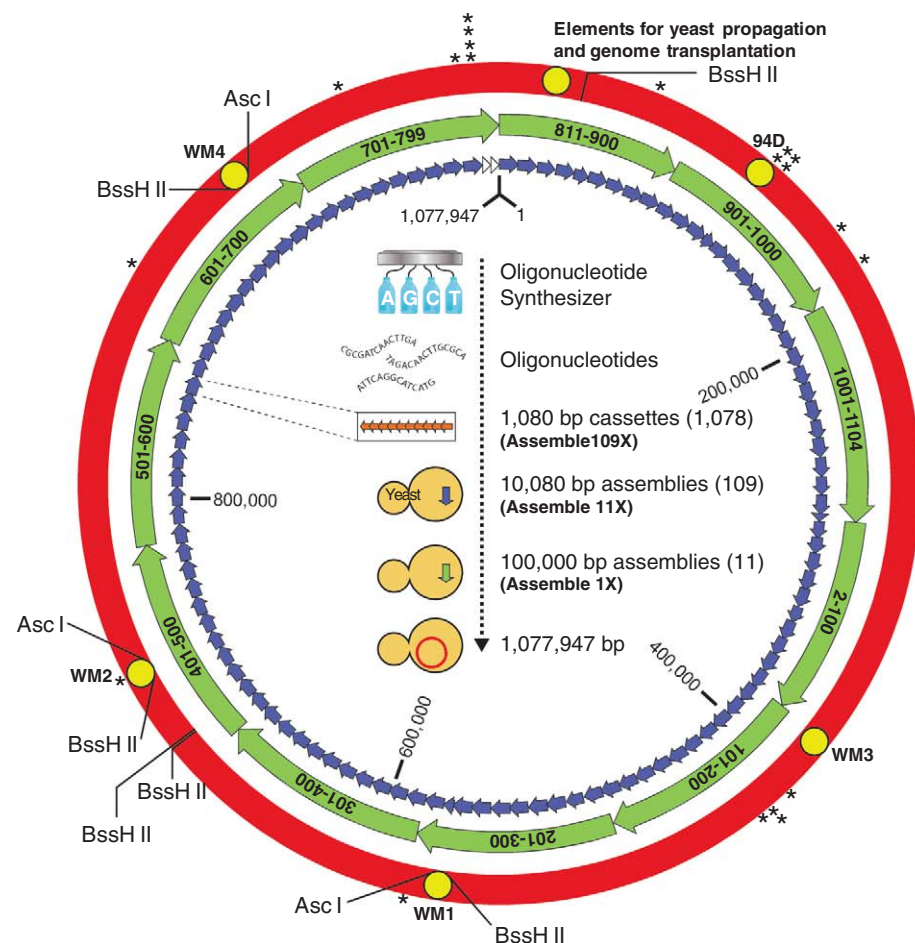


Fig. 1. The assembly of a synthetic *M. mycoides* genome in yeast. A synthetic *M. mycoides* genome was assembled from 1078 overlapping DNA cassettes in three steps. In the first step, 1080-bp cassettes (orange arrows), produced from overlapping synthetic oligonucleotides, were recombined in sets of 10 to produce 109 ~10-kb assemblies (blue arrows). These were then recombined in sets of 10 to produce 11 ~100-kb assemblies (green arrows). In the final stage of assembly, these 11 fragments were recombined into the complete genome (red circle). With the exception of two constructs that were enzymatically pieced together in vitro (27) (white arrows), assemblies were carried out by in vivo homologous recombination in yeast. Major variations from the natural genome are shown as yellow circles. These include four watermarked regions (WM1 to WM4), a 4-kb region that was intentionally deleted (94D), and elements for growth in yeast and genome transplantation. In addition, there are 20 locations with nucleotide polymorphisms (asterisks). Coordinates of the genome are relative to the first nucleotide of the natural *M. mycoides* sequence. The designed sequence is 1,077,947 bp. The locations of the Asc I and BssH II restriction sites are shown. Cassettes 1 and 800-810 were unnecessary and removed from the assembly strategy (11). Cassette 2 overlaps cassette 1104, and cassette 799 overlaps cassette 811.

above to produce 100-kb assembly intermediates (11). Our results indicated that these products cannot be stably maintained in *E. coli*, so recombined DNA had to be extracted from yeast. Multiplex polymerase chain reaction (PCR) was performed on selected yeast clones (fig. S3 and table S2). Because every 10-kb assembly intermediate was represented by a primer pair in this analysis, the presence of all amplicons would suggest an assembled 100-kb intermediate. In general, 25% or more of the clones screened contained all of the amplicons expected for a complete assembly. One of these clones was selected for further screening. Circular plasmid DNA was extracted and sized on an agarose gel alongside a supercoiled marker. Successful second-stage assemblies with the vector sequence are ~105 kb in length (Fig. 2B). When all amplicons were produced following multiplex PCR, a second-stage assembly intermediate of the correct size was usually produced. In some cases, however, small deletions occurred. In other instances, multiple 10-kb fragments were assembled, which produced a larger second-stage assembly intermediate. Fortunately, these differences could easily be detected on an agarose gel before complete genome assembly.

Complete genome assembly. In preparation for the final stage of assembly, it was necessary to isolate microgram quantities of each of the 11 second-stage assemblies (11). As reported (14), circular plasmids the size of our second-stage assemblies could be isolated from yeast spheroplasts after an alkaline-lysis procedure. To further purify the 11 assembly intermediates, they were treated with exonuclease and passed through an anion-exchange column. A small fraction of the total plasmid DNA (1/100) was digested with Not I and analyzed by field-inversion gel electrophoresis (FIGE) (Fig. 2C). This method produced ~1 µg of each assembly per 400 ml of yeast culture (~10¹¹ cells).

The method above does not completely remove all of the linear yeast chromosomal DNA, which we found could substantially decrease the yeast transformation and assembly efficiency. To further enrich for the 11 circular assembly intermediates, ~200 ng samples of each assembly were pooled and mixed with molten agarose. As the agarose solidifies, the fibers thread through and topologically “trap” circular DNA (15). Untrapped linear DNA can then be separated out of the agarose plug by electrophoresis, thus enriching for the trapped circular molecules. The 11 circular assembly intermediates were digested with Not I so that the inserts could be released. Subsequently, the fragments were extracted from the agarose plug, analyzed by FIGE (Fig. 2D), and transformed into yeast spheroplasts (11). In this third and final stage of assembly, an additional vector sequence was not required because the yeast cloning elements were already present in assembly 811-900.

To screen for a complete genome, multiplex PCR was carried out with 11 primer pairs,

designed to span each of the 11 100-kb assembly junctions (table S3). Of 48 colonies screened, DNA extracted from one clone (sMmYCp235) produced all 11 amplicons. PCR of the wild-type positive control (YCpMmyc1.1) produced an indistinguishable set of 11 amplicons (Fig. 3A). To further demonstrate the complete assembly of a synthetic *M. mycoides* genome, intact DNA was isolated from yeast in agarose plugs and subjected to two restriction analyses: Asc I and BssH II (11). Because these restriction sites are present in three of the four watermark sequences, this choice of digestion produces restriction patterns that are distinct from that of the natural *M. mycoides* genome (Figs. 1 and 3B). The sMmYCp235 clone produced the restriction pattern expected for a completely assembled synthetic genome (Fig. 3C).

Synthetic genome transplantation. Additional agarose plugs used in the gel analysis above (Fig. 3C) were also used in genome transplantation experiments (11). Intact synthetic *M. mycoides* genomes from the sMmYCp235 yeast clone were transplanted into restriction-minus *M. capricolum* recipient cells, as described (8). Results were scored by selecting for growth of blue colonies on SP4 medium containing tetracycline and X-gal at 37°C. Genomes isolated from this yeast clone produced 5 to 15 tetracycline-resistant blue colonies per agarose plug, a number comparable to that produced by the YCpMmyc1.1 control. Recovery of colonies in all transplantation experiments was dependent on the presence of both *M. capricolum* recipient cells and an *M. mycoides* genome.

Semisynthetic genome assembly and transplantation. To aid in testing the functionality of each 100-kb synthetic segment, semisynthetic genomes were constructed and transplanted. By mixing natural pieces with synthetic ones, the successful construction of each synthetic 100-kb assembly could be verified without having to sequence these intermediates. We cloned 11 overlapping natural 100-kb assemblies in yeast by using a previously described method (16). In 11 parallel reactions, yeast cells were cotransformed with fragmented *M. mycoides* genomic DNA (YCpMmyc 1.1) that averaged ~100 kb in length and a PCR-amplified vector designed to overlap the ends of the 100-kb inserts. To maintain the appropriate overlaps so that natural and synthetic fragments could be recombined, the PCR-amplified vectors were produced via primers with the same 40-bp overlaps used to clone the 100-kb synthetic assemblies. The semisynthetic genomes that were constructed contained between 2 and 10 of the 11 100-kb synthetic subassemblies (Table 1). The production of viable colonies produced after transplantation confirmed that the synthetic fraction of each genome contained no lethal mutations. Only one of the 100-kb subassemblies, 811-900, was not viable.

Initially, an error-containing 811-820 clone was used to produce a synthetic genome that did not transplant. This was expected because the error was a single-base pair deletion that creates a

frameshift in *dnaA*, an essential gene for chromosomal replication. We were previously unaware of this mutation. By using a semisynthetic genome construction strategy, we pinpointed 811-900 as the source for failed synthetic transplantation experiments. Thus, we began to reassemble an error-free 811-900 assembly, which was used to

produce the sMmYCp235 yeast strain. The *dnaA*-mutated genome differs by only one nucleotide from the synthetic genome in sMmYCp235. This genome served as a negative control in our transplantation experiments. The *dnaA* mutation was also repaired at the 811-900 level by genome engineering in yeast (17). A repaired 811-900

Fig. 2. Analysis of the assembly intermediates. (A) Not I and Sbf I double restriction digestion analysis of assembly 341-350 purified from *E. coli*. These restriction enzymes release the vector fragments (5.5 and 3.4 kb) from the 10-kb insert. Insert DNA was separated from the vector DNA on a 0.8% E-gel (Invitrogen). M indicates the 1-kb DNA ladder (New England Biolabs; NEB). (B) Analysis of assembly 501-600 purified from yeast. The 105-kb circles (100-kb insert plus 5-kb vector) were separated from the linear yeast chromosomal DNA on a 1% agarose gel by applying 4.5 V/cm for 3 hours. S indicates the BAC-Tracker supercoiled DNA ladder (Epicentre). (C) Not I restriction digestion analysis of the 11 ~100-kb assemblies purified from yeast. These DNA fragments were analyzed by FGE on a 1% agarose gel. The expected insert size for each assembly is indicated. λ indicates the lambda ladder (NEB). (D) Analysis of the 11 pooled assemblies shown in (C) following topological trapping of the circular DNA and Not I digestion. One-fortieth of the DNA used to transform yeast is represented.

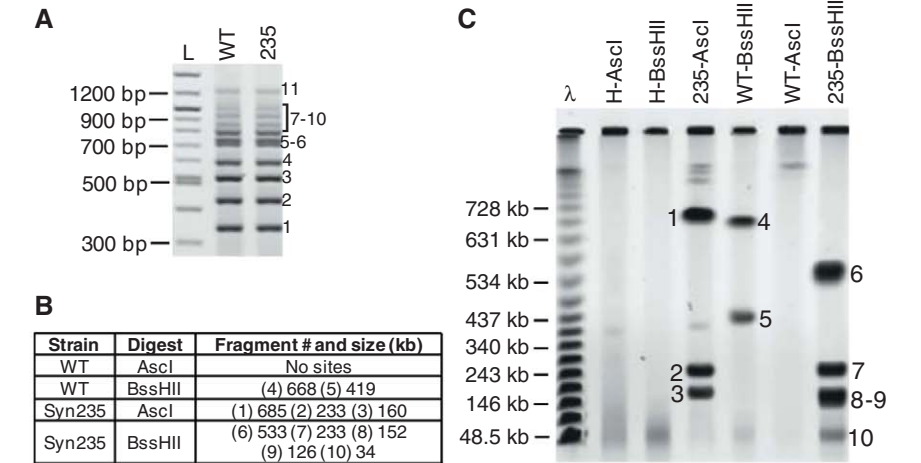
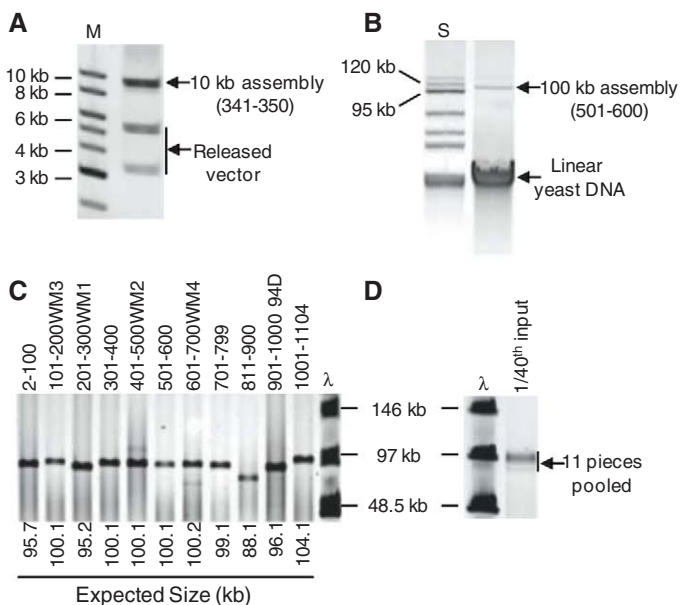


Fig. 3. Characterization of the synthetic genome isolated from yeast. (A) Yeast clones containing a completely assembled synthetic genome were screened by multiplex PCR with a primer set that produces 11 amplicons; one at each of the 11 assembly junctions. Yeast clone sMmYCp235 (235) produced the 11 PCR products expected for a complete genome assembly. For comparison, the natural genome extracted from yeast (WT, wild type) was also analyzed. PCR products were separated on a 2% E-gel (Invitrogen). L indicates the 100-bp ladder (NEB). (B) The sizes of the expected Asc I and BssH II restriction fragments for natural (WT) and synthetic (Syn235) *M. mycoides* genomes. (C) Natural (WT) and synthetic (235) *M. mycoides* genomes were isolated from yeast in agarose plugs. In addition, DNA was purified from the host strain alone (H). Agarose plugs were digested with Asc I or BssH II, and fragments were separated by clamped homogeneous electrical field (CHEF) gel electrophoresis. Restriction fragments corresponding to the correct sizes are indicated by the fragment numbers shown in (B).

assembly was used in a final-stage assembly to produce a yeast clone with a repaired genome. This yeast clone is named sMmYCP142 and could be transplanted. A complete list of genomes that have been assembled from 11 pieces and successfully transplanted is provided in Table 1.

Characterization of the synthetic transplants.

To rapidly distinguish the synthetic transplants from *M. capricolum* or natural *M. mycoides*, two analyses were performed. First, four primer pairs that are specific to each of the four watermarks were designed such that they produce four amplicons in a single multiplex PCR reaction (table S4). All four amplicons were produced by transplants generated from sMmYCP235, but not YCPmmyc1.1 (Fig. 4A). Second, the gel analysis with Asc I and BssH II, described above (Fig. 3C), was performed. The restriction pattern obtained was consistent with a transplant produced from a synthetic *M. mycoides* genome (Fig. 4B).

A single transplant originating from the sMmYCP235 synthetic genome was sequenced. We refer to this strain as *M. mycoides* JCVI-syn1.0. The sequence matched the intended design with the exception of the known polymorphisms, eight new single-nucleotide polymorphisms, an *E. coli* transposon insertion, and an 85-bp duplication (table S1). The transposon insertion exactly matches the size and sequence of IS1, a transposon in *E. coli*. It is likely that IS1 infected the 10-kb subassembly following its transfer to *E. coli*. The IS1 insert is flanked by direct repeats of *M. mycoides* sequence, suggesting that it was inserted by a transposition mechanism. The 85-bp duplication is a result of a nonhomologous end joining event, which was not detected in our sequence analysis at the 10-kb stage. These two insertions disrupt two genes that are evidently nonessential. We did not find any sequences in the synthetic genome that could be identified as belonging to *M. capricolum*. This indicates that there

was a complete replacement of the *M. capricolum* genome by our synthetic genome during the transplant process.

The cells with only the synthetic genome are self-replicating and capable of logarithmic growth. Scanning and transmission electron micrographs (EMs) of *M. mycoides* JCVI-syn1.0 cells show small, ovoid cells surrounded by cytoplasmic membranes (Fig. 5, C to F). Proteomic analysis of *M. mycoides* JCVI-syn1.0 and the wild-type control (YCPmmyc1.1) by two-dimensional gel electrophoresis revealed almost identical patterns of protein spots (fig. S4) that differed from those previously reported for *M. capricolum* (10). Fourteen genes are deleted or disrupted in the *M. mycoides* JCVI-syn1.0 genome; however, the rate of appearance of colonies on agar plates and the colony morphology are similar (compare Fig. 5, A and B). We did observe slight differences in the growth rates in a color-changing unit assay, with the JCVI-syn1.0 transplants growing slightly faster than the MmcyYCP1.1 control strain (fig. S6).

Discussion. In 1995, the quality standard for sequencing was considered to be one error in 10,000 bp, and the sequencing of a microbial genome required months. Today, the accuracy is substantially higher. Genome coverage of 30 to 50× is not unusual, and sequencing only requires a few days. However, obtaining an error-free genome that could be transplanted into a recipient cell to create a new cell controlled only by the synthetic genome was complicated and required many quality-control steps. Our success was thwarted for many weeks by a single-base pair deletion in the essential gene *dnaA*. One wrong base out of more than 1 million in an essential gene rendered the genome inactive, whereas major genome insertions and deletions in nonessential parts of the genome had no observable effect on viability. The demonstration that our synthetic genome gives rise to transplants with the characteristics of *M. mycoides* cells implies that the DNA sequence on which it is based is accurate enough to specify a living cell with the appropriate properties.

Our synthetic genomic approach stands in sharp contrast to various other approaches to genome engineering that modify natural genomes by introducing multiple insertions, substitutions, or deletions (18–22). This work provides a proof of principle for producing cells based on computer-designed genome sequences. DNA sequencing of a cellular genome allows storage of the genetic instructions for life as a digital file. The synthetic genome described here has only limited modifications from the naturally occurring *M. mycoides* genome. However, the approach we have developed should be applicable to the synthesis and transplantation of more novel genomes as genome design progresses (23).

We refer to such a cell controlled by a genome assembled from chemically synthesized pieces of DNA as a “synthetic cell,” even though the cytoplasm of the recipient cell is not synthetic. Phenotypic effects of the recipient cytoplasm are diluted with protein turnover and as cells carrying only the

Table 1. Genomes that have been assembled from 11 pieces and successfully transplanted. Assembly 2-100, 1; assembly 101-200, 2; assembly 201-300, 3; assembly 301-400, 4; assembly 401-500, 5; assembly 501-600, 6; assembly 601-700, 7; assembly 701-799, 8; assembly 811-900, 9; assembly 901-1000, 10; assembly 1001-1104, 11. WM, watermarked assembly.

Genome assembly	Synthetic fragments	Natural fragments
Reconstituted natural genome	None	1–11
2/11 semisynthetic genome with one watermark	5 WM, 10	1–4, 6–9, 11
8/11 semisynthetic genome without watermarks	1–4, 6–8, 11	5, 9, 10
9/11 semisynthetic genome without watermarks	1–4, 6–8, 10–11	5, 9
9/11 semisynthetic genome with three watermarks	1, 2 WM, 3 WM, 4, 6, 7 WM, 8, 10–11	5, 9
10/11 semisynthetic genome with three watermarks	1, 2 WM, 3 WM, 4, 5 WM, 6, 7 WM, 8, 10–11	9
11/11 synthetic genome, 811-820 correction of <i>dnaA</i>	1, 2 WM, 3 WM, 4, 5 WM, 6, 7 WM, 8, 9–11	None
11/11 synthetic genome, 811-900 correction of <i>dnaA</i>	1, 2 WM, 3 WM, 4, 5 WM, 6, 7 WM, 8, 9–11	None

Fig. 4. Characterization of the transplants. (A) Transplants containing a synthetic genome were screened by multiplex PCR with a primer set that produces four amplicons, one internal to each of the four watermarks. One transplant (syn1.0) originating from yeast clone sMmYCP235 was analyzed alongside a natural, nonsynthetic genome (WT) transplanted out of yeast. The transplant containing the synthetic genome produced the four PCR products, whereas the WT genome did not produce any. PCR products were separated on a 2% E-gel (Invitrogen). (B) Natural (WT) and synthetic (syn1.0) *M. mycoides* genomes were isolated from *M. mycoides* transplants in agarose plugs. Agarose plugs were digested with Asc I or BssH II and fragments were separated by CHEF gel electrophoresis. Restriction fragments corresponding to the correct sizes are indicated by the fragment numbers shown in Fig. 3B.

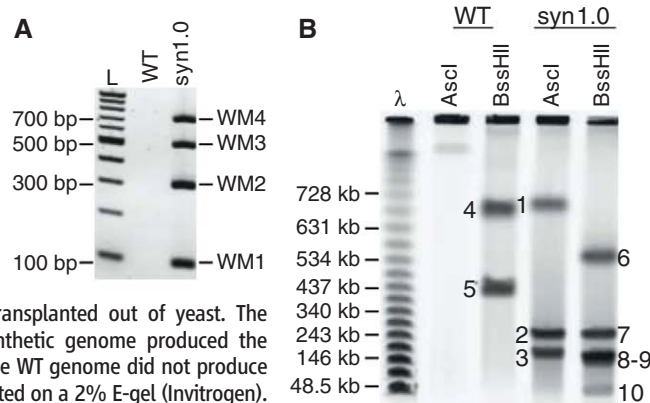
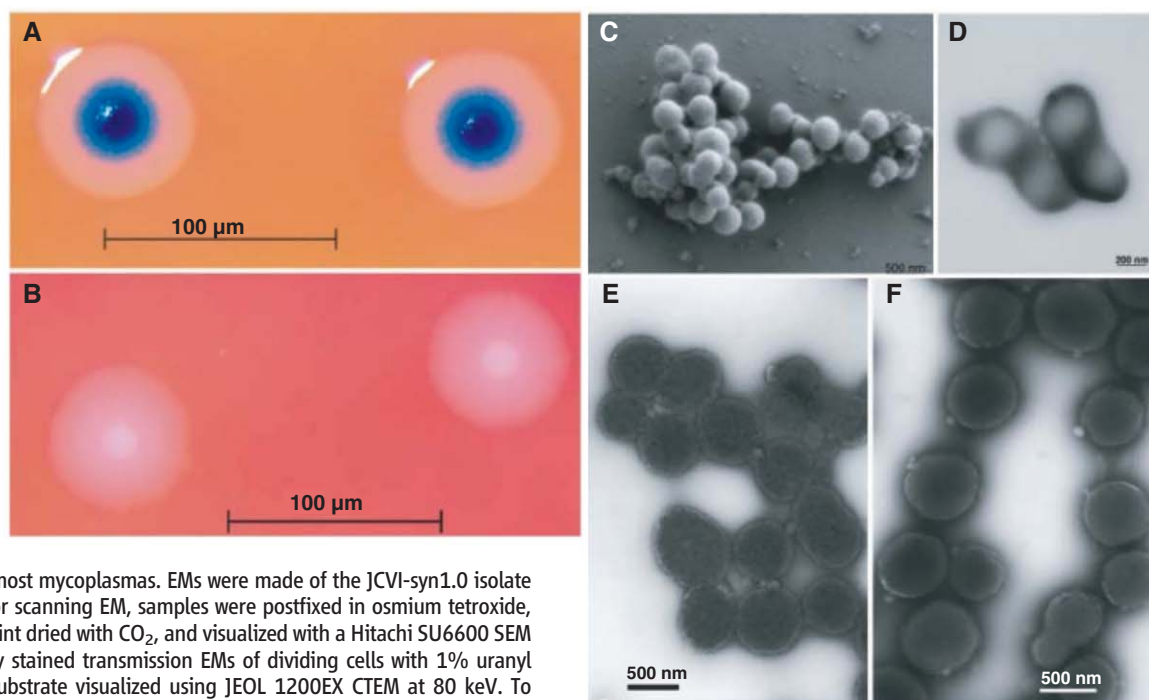


Fig. 5. Images of *M. mycoides* JCVI-syn1.0 and WT *M. mycoides*. To compare the phenotype of the JCVI-syn1.0 and non-YCp WT strains, we examined colony morphology by plating cells on SP4 agar plates containing X-gal. Three days after plating, the JCVI-syn1.0 colonies are blue because the cells contain the *lacZ* gene and express β -galactosidase, which converts the X-gal to a blue compound (A). The WT cells do not contain *lacZ* and remain white (B). Both cell types have the fried egg colony morphology characteristic of most mycoplasmas. EMs were made of the JCVI-syn1.0 isolate using two methods. (C) For scanning EM, samples were postfixed in osmium tetroxide, dehydrated and critical point dried with CO₂, and visualized with a Hitachi SU6600 SEM at 2.0 keV. (D) Negatively stained transmission EMs of dividing cells with 1% uranyl acetate on pure carbon substrate visualized using JEOL 1200EX CTEM at 80 keV. To examine cell morphology, we compared uranyl acetate-stained EMs of *M. mycoides* JCVI-syn1.0 cells (E) with EMs of WT cells made in 2006 that were stained with ammonium molybdate (F). Both cell types show the same ovoid morphology and general appearance. EMs were provided by T. Deerinck and M. Ellisman of the National Center for Microscopy and Imaging Research at the University of California at San Diego.



transplanted genome replicate. Following transplantation and replication on a plate to form a colony (>30 divisions or $>10^9$ -fold dilution), progeny will not contain any protein molecules that were present in the original recipient cell (10, 24). This was previously demonstrated when we first described genome transplantation (10). The properties of the cells controlled by the assembled genome are expected to be the same as if the whole cell had been produced synthetically (the DNA software builds its own hardware).

The ability to produce synthetic cells renders it essential for researchers making synthetic DNA constructs and cells to clearly watermark their work to distinguish it from naturally occurring DNA and cells. We have watermarked the synthetic chromosome in this and our previous study (7).

If the methods described here can be generalized, design, synthesis, assembly, and transplantation of synthetic chromosomes will no longer be a barrier to the progress of synthetic biology. We expect that the cost of DNA synthesis will follow what has happened with DNA sequencing and continue to exponentially decrease. Lower synthesis costs combined with automation will enable broad applications for synthetic genomics.

We have been driving the ethical discussion concerning synthetic life from the earliest stages of this work (25, 26). As synthetic genomic applications expand, we anticipate that this work will continue to raise philosophical issues that have broad societal and ethical implications. We encourage the continued discourse.

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24. A mycoplasma cell, with a mass of about 10^{-13} g, contains fewer than 10^5 molecules of protein. (If it contains 20% protein, this is equivalent to 2×10^{-14} g of protein per cell. At a molecular mass of 120 daltons per amino acid residue, each cell contains $(2 \times 10^{-14})/120 = 1.7 \times 10^{-16}$ mol of peptide residues. This is equivalent to $(1.7 \times 10^{-16}) \times (6 \times 10^{23}) = 1 \times 10^8$ residues per cell. If the average size of a protein is 300 residues, then a cell contains about 3×10^5 protein molecules.) After 20 cell divisions the number of progeny exceeds the total number of protein molecules in the recipient cell. So, following transplantation and replication to form a colony on a plate, most cells will contain no protein molecules that were present in the original recipient cell.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1190719/DC1
Materials and Methods
Figs. S1 to S6
Tables S1 to S7
References

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A Giant Planet Imaged in the Disk of the Young Star β Pictoris

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Here, we show that the ~ 10 -million-year-old β Pictoris system hosts a massive giant planet, β Pictoris b, located 8 to 15 astronomical units from the star. This result confirms that gas giant planets form rapidly within disks and validates the use of disk structures as fingerprints of embedded planets. Among the few planets already imaged, β Pictoris b is the closest to its parent star. Its short period could allow for recording of the full orbit within 17 years.

As giant planets form from dusty gas-rich disks that surround young stars through processes that are not completely understood. Two general mechanisms of such planets have been identified (1): (i) disk fragmentation and (ii) accretion of gas onto a solid, typically with a 5 to 10 Earth-mass (MEarth) core. Currently, available models do not offer a detailed description of all of the physical and dynamical steps involved in these processes. The lifetime of gas-rich disks limits the availability of nebular gas and, thus, defines the time window in which gas giant planets can form. Once formed, giant planets are predicted to interact with the disk and distort it, possibly leading to characteristic disk structures that can be used to infer the presence of planets and to constrain their orbits. Up to now, most giant planets have been detected around stars that are several orders of magnitude older than the lifetime of gas-rich circumstellar disks, preventing the validation of models of disk-planet interactions and the final phases of giant planet accretion.

The young $\sim 12^{+8}_{-4}$ million years (My), nearby to the Sun (and, consequently, to Earth) (19.3 ± 0.2 pc), 1.75-solar-mass (MSun) star β Pictoris (2, 3) hosts a wide (several hundreds of astronomical units), tenuous edge-on circumstellar dust disk (4). It is composed of dust particles continuously replenished through collisions of larger solid bodies (planetesimals, comets) and is referred to as a debris disk (5, 6), in contrast to more massive gas-rich counterparts around younger (ages of a few million years) stars. This disk has been studied in great detail over the past 25 years. Observations at optical to

the thermal infrared wavelengths revealed multiple disk structures (7–9), as well as asymmetries in disk size, scale height, and surface-brightness distributions (10, 11).

Some of these structures and asymmetries have been theoretically linked to the presence of one or more massive planets. An inner warp in the disk plane (12, 13), in particular, can be reproduced by detailed models that include a planetary-mass companion (13, 14). In addition, spectroscopic observations over several years revealed sporadic high-velocity infall of ionized gas to the star, attributed to the evaporation of cometlike bodies grazing the star (5, 15, 16). The observed comet infall has been attributed to the gravitational perturbations by a giant planet within the disk (17). Taken together, these data and models suggest that the β Pictoris disk is populated by dust, gas, solid kilometer-sized bodies, and possibly one or more planets.

Near-infrared images of β Pictoris obtained in 2003 (18) show a faint (apparent magnitude $L' = 11.2$ mag) pointlike source at ~ 8 astronomical units (AU) in projected separation from the star, within the northeast side of the dust disk. However, these data were not sufficient to determine whether this source was a gravitationally bound companion or an unrelated background star, whose projected position in the plane of the sky

happened to be close to β Pictoris. Further observations in January and February 2009 did not detect the companion candidate (19, 20), an outcome fully consistent with the proper motion of β Pictoris with respect to a background star and with the orbital motion of a physically bound companion.

Here, we present high-contrast and high-spatial-resolution near-infrared images obtained in October, November, and December 2009 with the European Southern Observatory's Very Large Telescope's (VLT) Adaptive Optics NaCo instrument (21, 22) [see the supporting online material (SOM) for more details on the observations and data reduction]. The images obtained in October 2009 (Fig. 1) show a faint point source southwest of the star, with a brightness ($\Delta L = L^* - L = 7.8 \pm 0.3$ mag, where L^* is the apparent L-band magnitude of the star and L is the apparent L-band magnitude of the planet) comparable to that ($\Delta L = L^* - L = 7.7 \pm 0.3$) of the source-detected northeast side of β Pictoris in November 2003 (Fig. 1). The source lies at a projected separation of 297.6 ± 16.3 milli-arc second (mas) and at a position angle (PA) of $210.6 \pm 3.6^\circ$. Within the error bars, the source is located in the plane of the disk. To confirm the signal detected southwest of β Pictoris in October 2009, we gathered further data in November and December 2009. Together, these data confirm the detection made in October 2009 (see SOM).

The images show that the source detected in November 2003 could not have been a background object (Fig. 2). If it was a background object, given the star's proper motion (table S1, SOM), the November 2003 source would be located and detectable 5.1 AU away, southeast (PA = 147.5°) of β Pictoris in the fall of 2009. The data do not show such a source (fig. S2). On the contrary, the source position in fall 2009 is compatible with the projected position in November 2003 if the source is gravitationally bound to the star (see below).

Based on the system age, distance, and apparent brightness of the companion, the widely used Baraffe *et al.* (23) evolutionary models predict a mass of $\sim 9 \pm 3$ Jupiter masses (M_{Jup}).

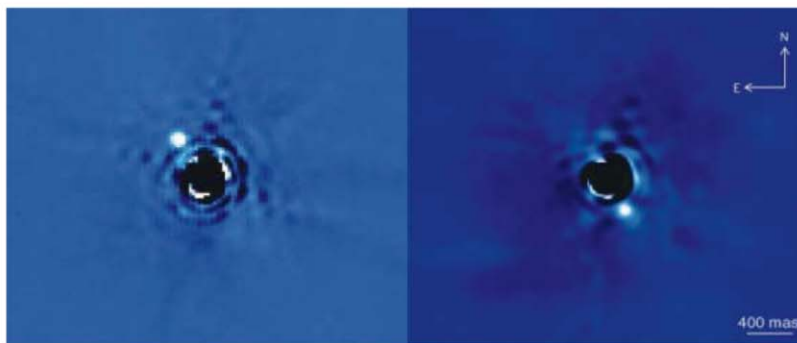


Fig. 1. β Pictoris imaged at L' band (3.78 microns) with the VLT/NaCo instrument in November 2003 (left) and the fall of 2009 (right). We used images of the comparison star HR2435 to estimate and remove the stellar halo (see SOM). Similar results are obtained when using angular differential imaging (see SOM).

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This value is compatible with those derived from the calculations of other groups (24), when assuming, as did Baraffe *et al.* (23), that the planets form from the spherical contraction and cooling of a hot, initially nonrotating cloud of gas. However, it is not clear whether the basic assumption of these “hot-start” models (that is, the spherical contraction) applies to β Pictoris b or other planets. A recently developed, leading alternative model (24) includes the loss of energy of the infalling gas via an accretion shock (core accretion start), resulting in the accretion of cooler gas to the forming planet. This “cold-start” model predicts luminosities that are two orders of magnitudes lower than those predicted by the hot-start models at young (~ 10 My) ages for a $\sim 10 M_{\text{Jup}}$ -mass planet. The initial difference between the two models decreases after formation; however, it remains very large for these massive planets, even at ages of 100 My (factor of 10). The low luminosities predicted by the cold-start model are not easy to reconcile with the brightness of β Pictoris b. This apparent inconsistency suggests that this model overestimates the energy lost during the formation of β Pictoris b. The assumptions underlying the hot-start or cold-start models are still a matter of debate, and observations of objects such as β Pictoris b are essential to test them. Hereafter, we use the mass inferred by the hot-start model, because there are lines of evidence that the companion cannot be much more massive (see below).

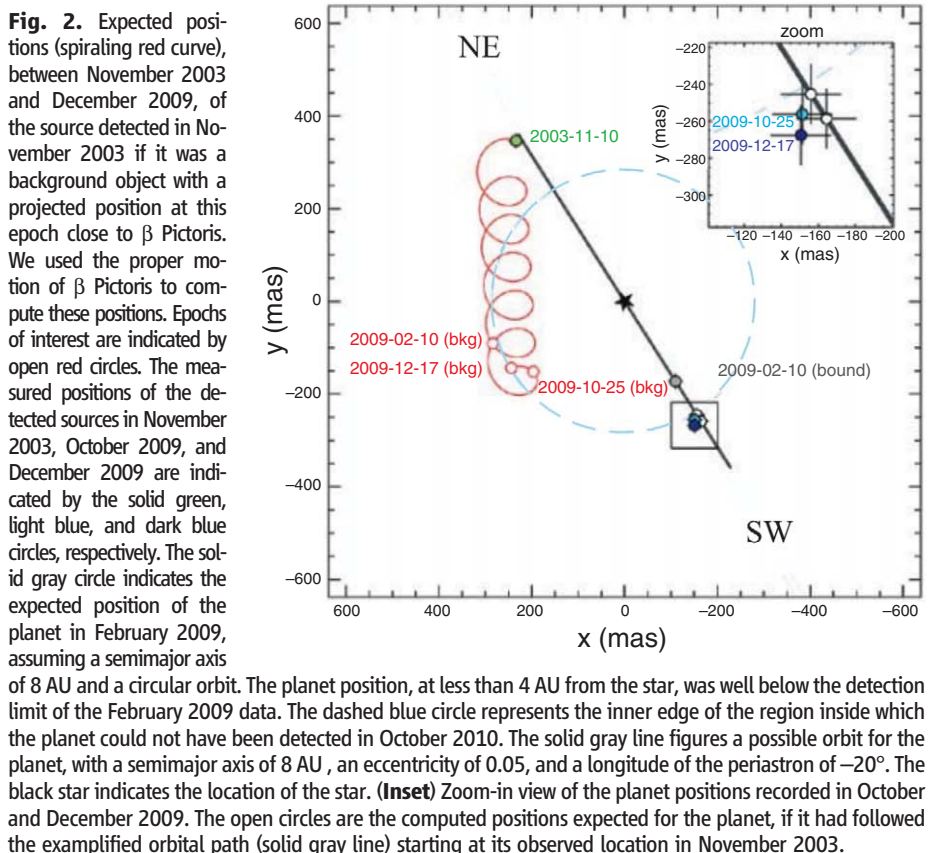
To constrain the orbital parameters of β Pictoris b, we took the projected separation measured in November 2003 and computed the expected position of the planet in 2009 (see SOM), assuming that it moves in a prograde [following (25)], circular orbit within the disk or close to the plane of the disk. A comparison between the expected projected separation in December 2009 and the observed position implies that the planet’s semimajor axis is between 8 and 13 AU (fig. S3), which leads to orbital periods of 17 to 35 years for the planet. The semimajor axis is less strongly constrained in the case of an eccentric orbit because of the unknown longitude of the periastron. However, for the probable case of a moderate eccentricity [$e < 0.05$ (26)], the semimajor axis must be in the range of 8 to 15 AU. These orbital parameters are compatible with the nondetection at L' band in February 2009 (Fig. 2), given the 4σ detection limits at this date that correspond to projected separations of 6.5 AU. Thus, β Pictoris b orbits closer to its parent star than Uranus and Neptune do in the solar system.

The planet separation is qualitatively consistent with the observation of beltlike structures in the inner disk at 6 ± 3 and 16 ± 3 AU (7, 8). The separation and mass are fully consistent with those predicted by dynamical studies that invoked a planet to reproduce the inner-disk warp (13, 14). On the contrary, a more massive ($> 40 M_{\text{Jup}}$) companion at such separations would not be compatible with the warp constraints (see SOM).

Finally, it has been suggested that the 2003 candidate planet could have been responsible for a peculiar photometric variability event observed in 1981, when transiting in front of the star (27). The 2009 data are compatible with this possibility [see also (19)], however, only for a small range of planet orbital parameters.

The detection of β Pictoris b follows on the recent detections of planets around the intermediate-mass stars HR 8799 and Fomalhaut [see SOM and (28, 29)]. These stars are also surrounded by debris disks (30, 31). However, β Pictoris b orbits closer to its star and is younger than the planets around HR 8799 and Fomalhaut (30 to 160 and 100 to 300 My old, respectively, tables S2A and S2B). Our images of β Pictoris b provide direct evidence that massive giant planets can form rapidly, on time scales of a few million years within circumstellar disks (32). This is in agreement with studies of the dispersion of primordial disks around young intermediate-mass stars, which yield typical disk lifetimes of between < 3 and 6 My (16).

A comparison of the luminosity of β Pictoris [$8.7 L_{\text{Sun}}$ (L_{Sun} , solar luminosity) (3)] to that of the Sun suggests that the orbit of β Pictoris b lies at or slightly beyond the disk radius outside which water is as stable as ice (snow line). The snow line is thought to separate disk regions where rocky or gaseous/icy planets form (33, 34). Beyond the snow line, the disk-surface density is expected to be higher (by a factor of 3) than that inside the line; this allows giant planet cores ($10 M_{\text{Earth}}$) to form before the dispersion of the gaseous nebulae. Core-accretion models suggest that this latter step—the onset of rapid gas accretion before the loss of circumstellar gas—is the critical step in forming giant planets. Assuming a core with a minimum mass of 5 to $10 M_{\text{Earth}}$ and taking into account the snow-line properties as a function of stellar mass and age, the model of Kennedy and Kenyon (33) determines the snow-line position as a function of time and possible locations of giant protoplanet cores as a function of stellar mass. For a $2 M_{\text{Sun}}$ star, the snow-line location varies between 2.5 and 4 AU for ages between 1 and 10 My, respectively. In the case of the $1.75 M_{\text{Sun}}$ β Pictoris, core-accretion-based models predict a rapid formation of giant protoplanet cores between ~ 6 and 18 AU. The observed orbital radius of β Pictoris b is consistent with this range, demonstrating that the planet could have formed via core accretion on the same orbit where it is observed today. This possibility is in contrast to the case of the more distant planets Fomalhaut b, HR8799bc, AB Pic b, and 2MASS 1207b, which are too massive (tables S2A and S2B) to have formed at their present separations (40 AU or larger) via core accretion.



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- However, its formation process (stellarlike or through gravitational instability within disk) is still a matter of debate.
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Controlled Injection of Spin-Triplet Supercurrents into a Strong Ferromagnet

J. W. A. Robinson,* J. D. S. Witt, M. G. Blamire

The superconductor-ferromagnet proximity effect describes the fast decay of a spin-singlet supercurrent originating from the superconductor upon entering the neighboring ferromagnet. After placing a conical magnet (holmium) at the interface between the two, we detected a long-ranged supercurrent in the ferromagnetic layer. The long-range effect required particular thicknesses of the spiral magnetically ordered holmium, consistent with spin-triplet proximity theory. This enabled control of the electron pairing symmetry by tuning the degree of magnetic inhomogeneity through the thicknesses of the holmium injectors.

The electronic properties of a material that has been cooled below its superconducting transition temperature are influenced by the pairing symmetry of the electrons. In a conventional superconductor, the Cooper pairs are formed from electrons with an antiparallel spin alignment and are in the spin-singlet state (1, 2). In contrast to superconductivity, ferromagnetism favors a parallel alignment of electron spins. Consequently, superconductivity and ferromagnetism rarely coexist, and diverse and complex phenomena arise at the interface between superconducting and ferromagnetic thin films (3). The most striking manifestation happens when spin-singlet Cooper pairs pass through a ferromagnet: The differential action of the ferromagnetic exchange field creates a spatially varying phase, which results in an oscillatory damping of the

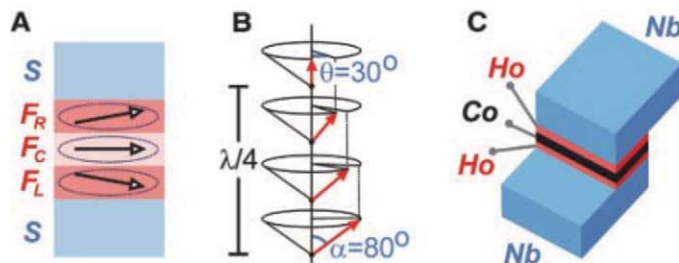
critical current (I_C) over a ferromagnetic thickness of a few nanometers (4–10).

Recent experiments have detected a longer-ranged effect in which the superconductivity appears to be insensitive to ferromagnetic exchange fields (11, 12). These results could be explained in the context of spin-triplet pairing in which Cooper pairs are formed with a parallel spin

alignment at the superconductor-ferromagnet interface (13–15). The spin-triplet pair is believed to be only weakly affected by the exchange field so that its phase coherence decays on the same length scale as that of spin-singlet pairs in a normal metal. Within this theoretical framework, the generation of spin-triplet electron pairs requires the presence of particular magnetic inhomogeneity at the superconductor-ferromagnet interface (13, 16).

Long-range Josephson coupling is presently the most robust way of detecting a spin-triplet current, and was reported in (11, 12) for a barrier formed from the half-metal CrO_2 . Supporting theory (14) suggested that the required magnetic inhomogeneity for the spin-triplet proximity effect could be provided by hypothetical spin disorder at the surface of the half-metal. A more recent theory (16) indicates that two matched spin-triplet sources are needed to achieve a Josephson effect; physically, this condition requires both interfaces to be magnetically noncollinear and to share specific symmetries. Because the nature of the inhomogeneity is uncertain in the CrO_2 -based junctions, reproducibly achieving these symmetry requirements in

Fig. 1. (A) Theoretical spin-triplet Josephson junction adapted from (16), consisting of two spin-singlet superconductors (S) linked via a noncollinear ferromagnetic trilayer (F_L - F_C - F_R). **(B)** The conical magnetic configuration of idealized Ho below its Curie temperature (20 K), showing an antiferromagnetic spiral rotating in-plane by $\theta = 30^\circ$ per atomic plane and pitched at $\alpha = 80^\circ$ out-of-plane. The moments (arrows) rotate about the surface of a cone with the spiral wavelength, λ , corresponding to a Ho thickness of ~ 3.4 nm. **(C)** Device layout consisting of two superconducting Nb electrodes coupled via a Ho-Co-Ho trilayer.



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this system is challenging. An enhanced proximity effect was also recently reported in (17); here the likely source of magnetic inhomogeneity was in secondary ferromagnet/normal metal bilayers placed at the superconductor/ferromagnet interface.

For a more straightforward interpretation of the results, an appealing approach would be to use one of the intrinsically inhomogeneous ferromagnets such as the rare earth metal Ho (18) coupled to a homogeneous ferromagnetic barrier. Long-range superconducting phase-coherent oscillations were reported in Ho wires (19) grown by evaporation and contacted inside a superconducting ring, but a Josephson current was not detected.

Our experiment was motivated by the proposal (16) for a spin-triplet Josephson junction consisting of two spin-singlet superconductors (S) coupled via a ferromagnetic trilayer ($F_L/F_C/F_R$). The magnetization of F_L and F_R layers should be noncollinear to provide the necessary inhomogeneity for the spin-triplet configuration of electron spins to be favorable (Fig. 1A). The experiment enables the decay length of the supercurrent from spin-triplet pairs in the homogeneous central ferromagnet F_C to be directly compared with that in simple homogeneous ferromagnetic barriers of the same material and thickness.

We report results from structures in which Ho was used for F_L and F_R , and Co was used for F_C (Fig. 1, B and C). The conical magnetic

ordering of Ho, which consists of an antiferromagnetic spiral canted to produce a net ferromagnetic component in the c -axis orientation, allows for the inclusion of reproducibly noncollinear magnetic layers within device structures. Moreover, its magnetic properties and a preferential (0001) texture are robust even in thin films at the nanometer scale (20).

We processed several series of nanoscale Nb/Ho/Co/Ho/Nb junctions with varying Ho and Co layer thicknesses (21); within each junction, the thicknesses of F_L and F_R Ho layers were equal and varied in the 0- to 12-nm range with an absolute error of ~ 0.2 nm. The electrical properties of these junctions were measured at 4.2 K, from which the critical current (I_C) and normal state resistance (R_N) of a device were determined (21). Because device areas varied, I_C was normalized by multiplying by R_N to give the characteristic voltage ($I_C R_N$).

The behavior of simple Co barrier junctions is well understood: The singlet-based I_C oscillates as a function of Co thickness with a period of ~ 1 nm superimposed on an exponentially decaying function with a characteristic length of $\xi_{Co} \sim 1$ nm [Fig. 2A, inset; data from Nb/Rh/Co/Rh/Nb junctions in (22)]. This structure was chosen because it represents an equivalent layering sequence with the same number of interfaces and therefore acts as a better control sample than a pure Nb/Co/Nb junction (which nevertheless shows similar properties).

The main plot in Fig. 2A shows the Co thickness dependence of $I_C R_N$ for Nb/Ho/Co/Ho/Nb junctions. In comparison with the Co barrier junctions, the decay length is substantially longer by a factor of at least 20. The figure shows an approximate fit (shaded region) giving a coherence length of $\xi_{Co} > 10$ nm, which agrees with the normal (nonmagnetic) coherence length $(\hbar D/k_B T)^{1/2} \sim 10$ nm assuming an electron diffusivity of $D \approx 4.3 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$ (and where $\hbar$ is Planck's constant h divided by 2π , k_B is Boltzmann's constant, and T is temperature) (22); that is, the supercurrent is passing through the composite Ho/Co/Ho barrier as if it were nonmagnetic.

To understand in more detail the role of the Ho layers, we symmetrically varied d_{Ho} [any asymmetry (Δd_{Ho}) < 0.2 nm] for several values of Co barrier thicknesses (Fig. 2B). In the 2-nm Co data, increasing the thickness of the Ho layers results in an increase in $I_C R_N$ of more than an order of magnitude, despite the overall increase in barrier thickness and total magnetic moment. Plain Co barriers of 5 and 8 nm show no measurable supercurrent in our previous experiments (9, 22). Further measurements confirming the presence of a Josephson effect are given in Fig. 3, A and B. External microwaves give rise to sharp dips in the dynamic resistance at particular voltage (V) values (Fig. 3A). These Shapiro steps occur at integer values of $V/\phi_0 f = \pm 1$, where ϕ_0 is the flux quantum and f is the applied microwave frequency. Upon application

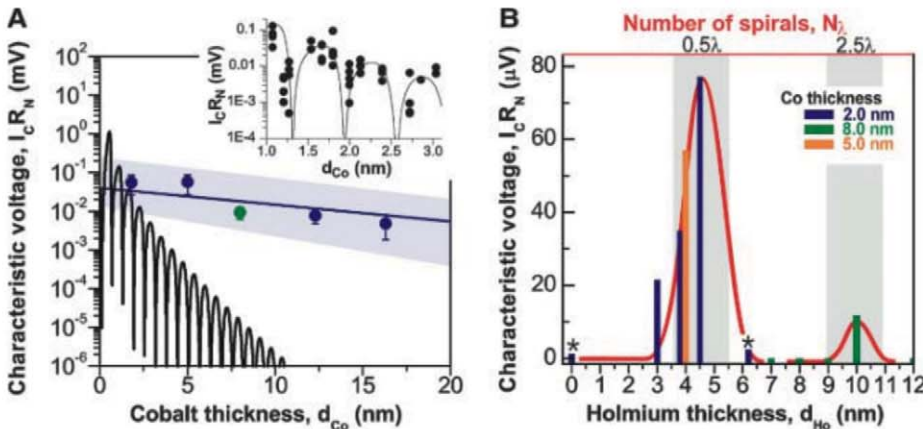


Fig. 2. (A) Slow decay at 4.2 K in the characteristic voltage of Nb/Ho(4.5 nm)/Co(d_{Co})/Ho(4.5 nm)/Nb junctions (blue circles) and Nb/Ho(10 nm)/Co(d_{Co})/Ho(10 nm)/Nb junctions (green circles) versus Co barrier thickness (d_{Co}). Inset: Comparative data (black circles) from (22) showing the behavior of Nb/Rh/Co/Rh/Nb junctions. The oscillating curves in the inset and main panel are theoretical fits to the experimental data in the inset, as described in (22). (B) Characteristic voltage in Nb/Ho(d_{Ho})/Co/Ho(d_{Ho})/Nb junctions at 4.2 K versus Ho layer thickness (d_{Ho}) for various Co barrier thicknesses. The thicknesses of each Ho layer in a junction are identical. The peaks correlate to noninteger spiral wavelengths (λ) in Ho. Asterisks identify small, but nonzero characteristic voltage values. The red curves are a guide to the eye.

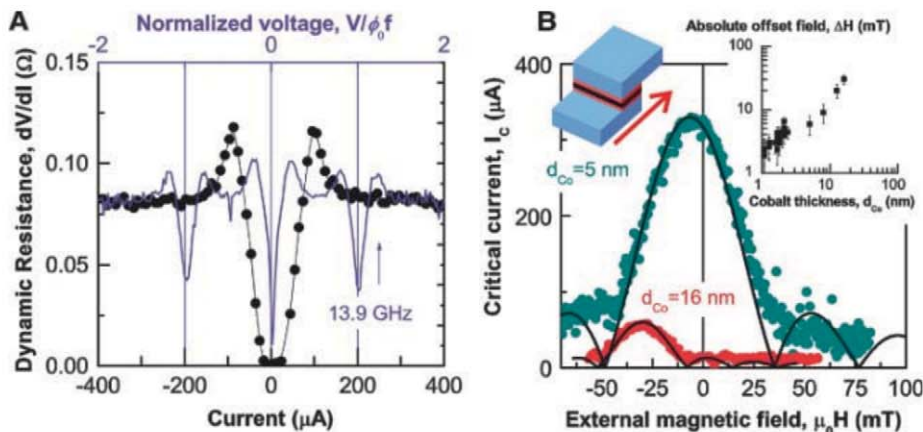


Fig. 3. (A) Dynamic resistance of a Nb/Ho(4.5 nm)/Co(16 nm)/Ho(4.5 nm)/Nb junction versus current and voltage at 4.2 K with and without microwaves. The normal state resistance of this device is $R_N \approx 0.076$ ohm and the critical current is $I_C \approx 90$ μA . The voltage scale is divided by $\phi_0 f$, where ϕ_0 is the flux quantum and f is the microwave frequency. A constant in-plane field of -32 mT was applied during these measurements to cancel out internal flux and demagnetizing fields from the Co barrier. (B) Critical current versus in-plane magnetic field at 4.2 K. The critical currents are offset in field (ΔH) due to internal flux and demagnetizing fields from the Co barrier. Solid curves are a guide to the eye. Insets: (left) illustration of a junction showing the field orientation, and (right) absolute ΔH versus Co barrier thickness.

of an external in-plane magnetic field (H) to our junctions, we observe a Fraunhofer-like dependence of I_C on H . The maximum I_C values are, however, offset from zero field (ΔH) due to the presence of internal flux and demagnetizing fields from the Co barriers. The absolute value of ΔH linearly depends on the Co barrier thickness (Fig. 3B inset), demonstrating that the Co barriers are monodomain in nature (21).

Taken together, the data in Fig. 2, A and B, show a complex variation of $I_C R_N$ over the thickness range investigated, with peaks corresponding to Ho thicknesses of ~ 4.5 and ~ 10 nm. By measuring the saturation magnetization of a series of Nb/Ho/Co/Ho/Nb control samples, we determined a magnetically “dead” layer of ~ 1.2 nm per Ho surface (21) (fig. S1A). Thus, the peaks in $I_C R_N$ in Fig. 2B correspond to magnetic Ho layer thicknesses of ~ 2.2 and ~ 7.8 nm, which are comparable to the experimentally determined coherence length in Ho of $\xi_{\text{Ho}} \sim 5$ nm (21). This is then broadly consistent with the analysis in (16), in which the largest spin-triplet contribution to I_C is predicted to occur when F_L and F_R layers have a thickness in the $(0.5 \text{ to } 2.5)\xi$ range. However, this cannot on its own explain the peak structure, and so we considered a possible link between the peak thicknesses and the known spiral wavelength of Ho, $\lambda \sim 3.4$ nm (23). Factoring in the magnetically dead layer of Ho implies that the peak values of $I_C R_N$ correspond to antiferromagnetic spiral wavelengths of $\sim \lambda/2$ and $\sim 5(\lambda/2)$. Although an exact parallel between these peaks and the magnetic ordering cannot

be drawn from this analysis, it is nevertheless clear that the peaks appear at thicknesses corresponding to a high level of inhomogeneity in the Ho, i.e., at thicknesses in which the spirals are incomplete.

The long-range effect reported cannot be explained in terms of a spin-singlet proximity theory or a complex domain-wall-related phenomenon (24, 25). A controllable supercurrent with a finite spin projection can allow for a more complete interaction between superconductivity and magnetism, possibly bringing together the previously disparate fields of superconductivity and spin-electronics (26, 27).

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Materials and Methods

Fig. S1

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Quantized Anomalous Hall Effect in Magnetic Topological Insulators

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The anomalous Hall effect is a fundamental transport process in solids arising from the spin-orbit coupling. In a quantum anomalous Hall insulator, spontaneous magnetic moments and spin-orbit coupling combine to give rise to a topologically nontrivial electronic structure, leading to the quantized Hall effect without an external magnetic field. Based on first-principles calculations, we predict that the tetradymite semiconductors Bi_2Te_3 , Bi_2Se_3 , and Sb_2Te_3 form magnetically ordered insulators when doped with transition metal elements (Cr or Fe), in contrast to conventional dilute magnetic semiconductors where free carriers are necessary to mediate the magnetic coupling. In two-dimensional thin films, this magnetic order gives rise to a topological electronic structure characterized by a finite Chern number, with the Hall conductance quantized in units of e^2/h (where e is the charge of an electron and h is Planck's constant).

The anomalous Hall effect (AHE) (1, 2), in which a voltage transverse to the electric current appears even in the absence of an external magnetic field, was first detected in ferromagnetic (FM) metals in 1881 and later found to arise from the spin-orbit coupling (SOC) between the current and magnetic moments. Recent progress on the mechanism of AHE has established a link between the AHE and the topological nature of the Hall current by adopting the

Berry-phase concepts (3–5) in close analogy to the intrinsic spin Hall effect (6, 7). Given the experimental discovery of the quantum Hall (8) and the quantum spin Hall (QSH) effects (9, 10), it is natural to ask whether the AHE can also be quantized.

A simple mechanism for a quantum anomalous Hall (QAH) insulator has been proposed in a two-band model of a two-dimensional (2D) magnetic insulator (11). In the limit of vanishing

SOC and large enough exchange splitting, the majority spin band is completely filled and the minority spin band is empty. When the exchange splitting is reduced, the two bands intersect each other, leading to a band inversion. The degeneracy at the interaction region can be removed by turning on the SOC, giving rise to an insulator state with a topologically nontrivial band structure characterized by a finite Chern number and chiral edge states characteristic of the QAH state (11). Alternative mechanisms of realizing the QAH state include bond currents on a honeycomb lattice (12) and the localization of the band electrons (13). However, these mechanisms may be harder to realize experimentally.

The crucial criteria for realizing a QAH state are (i) a FM 2D insulator that breaks the time-reversal symmetry and (ii) a band inversion transition with strong SOC. QSH insulators are a good starting point for the search for the QAH effect because they satisfy the second criterion.

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Magnetically doped HgMnTe has been proposed as a candidate for the QAH insulator (14); however, because the Mn moments do not order spontaneously in HgMnTe, an additional, small Zeeman field is required.

Recently, tetradymite semiconductors Bi₂Te₃, Bi₂Se₃, and Sb₂Te₃ have been theoretically predicted and experimentally observed to be topological insulators (TIs) with the bulk band gap as large as 0.3 eV in Bi₂Se₃ (15–17). As the thickness is reduced, a three-dimensional (3D) TI crosses over to a 2D TI in an oscillatory fashion (18). We predict that thin films made out of this family of compounds doped with proper transition metal

elements (Cr or Fe) support the QAH state. Recent experimental progress has shown that well-controlled layer-by-layer molecular beam epitaxial thin-film growth can be achieved (19, 20), and various transition metal elements (such as Ti, V, Cr, and Fe) can be substituted into the parent compounds with observable ferromagnetism even above 100 K (21–23).

We first discuss minimal requirements for the FM insulator phase in a semiconductor system doped with dilute magnetic ions under the assumption that the magnetic exchange among local moments is mediated by the band electrons. The whole system can then be divided into two

subsystems describing the local moments and band electrons, respectively, coupled by a magnetic exchange term. If we only consider the spatially homogeneous phase, the total free energy of the system in an external magnetic field H can be written as

$$F_{\text{total}} = \frac{1}{2}\chi_L^{-1}M_L^2 + \frac{1}{2}\chi_e^{-1}M_e^2 - J_{\text{eff}}M_LM_e - (M_L + M_e)H \quad (1)$$

where $\chi_{L/e}$ is the spin susceptibility of the local moments/electrons, $M_{L/e}$ denotes the magnetization for the local moment and electron subsystem, and J_{eff} is the magnetic exchange coupling between them. In order to have a nonzero FM transition temperature (T_c), a sizable χ_e is needed [for a detailed analysis, see (24)]. In most dilute magnetic semiconductors, for example, (Ga_{1-x}Mn_x)As, the electronic spin susceptibility is negligible for the insulator phase, and finite carrier concentration is required to mediate the magnetic coupling among local moments. The insulating Bi₂Se₃, however, gains considerable spin susceptibility through the Van Vleck paramagnetism (25), which is caused by the nonzero matrix element of the spin operator, S_z , between the valence and conduction bands (24). For the Bi₂Se₃ family, the semiconductor gap is opened by the SOC between the bonding and antibonding p orbitals, leading to large matrix elements (24). Such a mechanism is absent in the GaAs system, which has s -like conduction and p -like valence bands.

The Van Vleck type spin susceptibility can be further enhanced by band inversion. To demon-

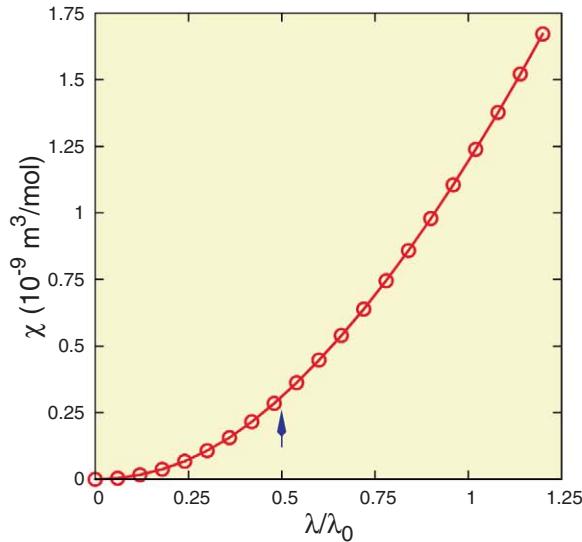
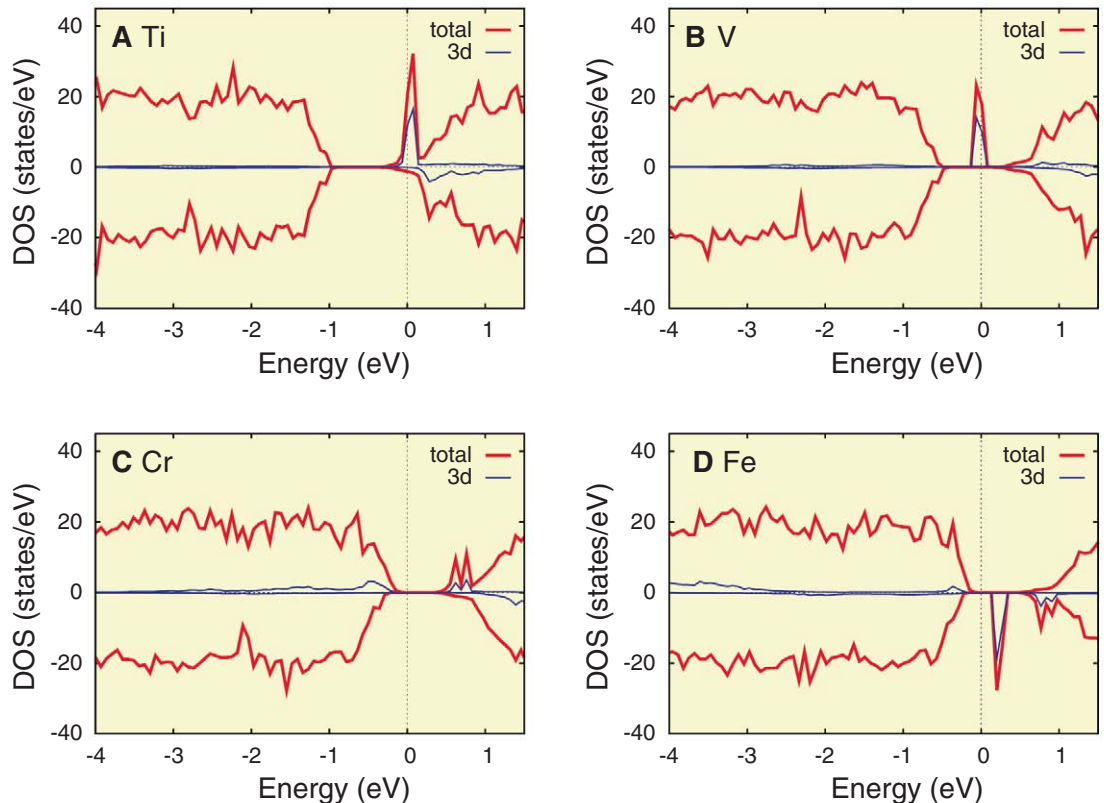


Fig. 1. The calculated spin susceptibility for Bi₂Se₃. The Van Vleck type spin susceptibility of Bi₂Se₃ bulk as a function of the SOC strength (λ_0 is the actual SOC strength).

Fig. 2. (A to D) The calculated density of states (DOS) for Bi₂Se₃ doped with different transition metal elements. The Fermi level is located at energy zero, and the positive and negative values of DOS are used for up and down spin, respectively. The blue lines are projected partial DOS of the 3d states of transition metal ions. It is shown that Cr or Fe doping will give rise to the insulating magnetic state.



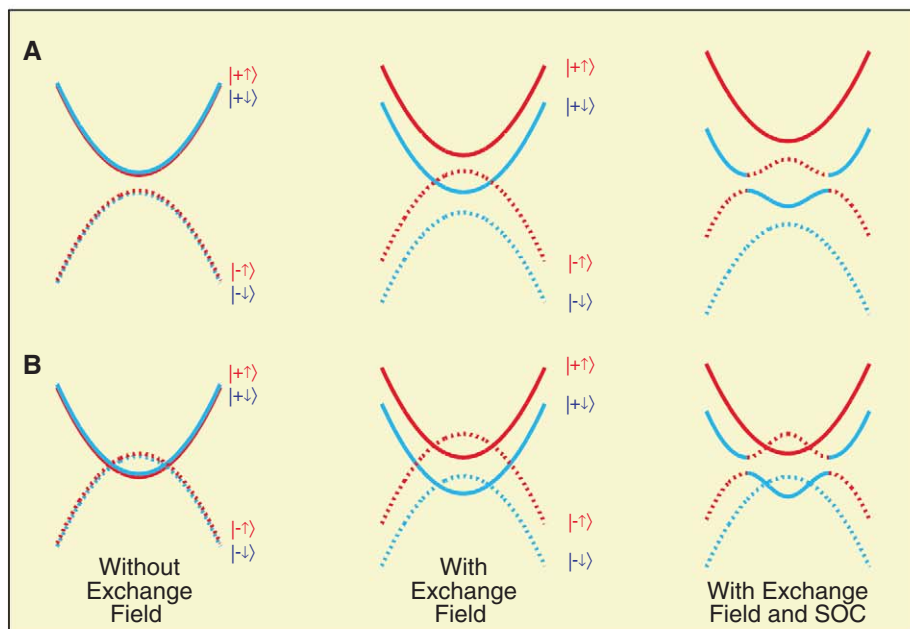


Fig. 3. Evolution of the subband structure upon increasing the exchange field. The solid lines denote the subbands that have even parity at Γ point, and dashed lines denote subbands with odd parity at Γ point. The blue color denotes the spin down electrons; red, spin up electrons. **(A)** The initial subbands are not inverted. When the exchange field is strong enough, a pair of inverted subbands appears (red dashed line and blue solid line). **(B)** The initial subbands are already inverted. The exchange field releases the band inversion in one pair of subbands (red solid line and blue dashed line) and increase the band inversion in the other pair (red dashed line and blue solid line).

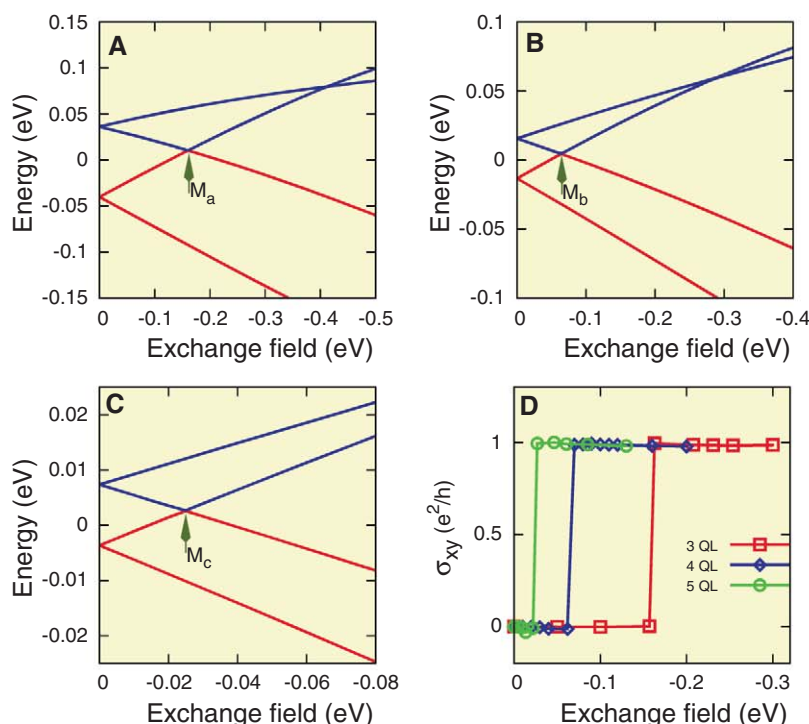


Fig. 4. The QAH conductance. **(A to C)** The lowest subbands at the Γ point plotted versus the exchange field, for Bi_2Se_3 films with thicknesses of three, four, and five quintuple layers (QL), respectively. The red lines denote the top occupied states; blue lines, bottom unoccupied states. With increasing exchange field, a level crossing occurs (arrows), indicating a quantum phase transition to QAH state. **(D)** The calculated Hall conductance for three, four, and five QL Bi_2Se_3 films versus the ferromagnetic exchange field. The Hall conductance is zero before the QAH transition but $\sigma_{xy} = e^2/h$ afterward.

strate this argument, we perform first-principles calculations (24) for the electronic structures and the spin susceptibility of Bi_2Se_3 bulk (Fig. 1). The band inversion at the Γ point occurs when the relative SOC strength λ/λ_0 exceeds 0.5, and the spin susceptibility starts to increase appreciably. The susceptibility tensor is anisotropic, with $\chi_e^{zz}/\chi_e^{xx} \approx 1.1$ estimated for the parent compound (without magnetic dopants). In the presence of dopants, the single-ion magnetic anisotropy will dominate and favor the off-plane orientation (23, 26).

We show, by first-principles calculations, that the insulating magnetic ground state discussed above can be indeed obtained by a proper choice of magnetic dopants. Experiments (21–23) have suggested that the magnetic dopants, such as Ti, V, Cr, and Fe, will mostly substitute the Bi ions; we therefore concentrate on this situation. Because the nominal valence of Bi ions is 3+, a general rule is to find a transition metal element that may have a stable 3+ chemical state, so that no free carriers are introduced by this isoelectronic substitution. In addition, because of the coexistence of orbital and spin degrees of freedom of magnetic dopants (due to the partially filled d shells), we need a mechanism to quench these degrees of freedom and stabilize the insulating state.

The results shown in Fig. 2 suggest that an insulating magnetic state is obtained for Cr or Fe doping, whereas the states are metallic for Ti or V doping cases (24). To understand the results, we first point out that, for all the cases, the dopants are nearly in the 3+ valence state, and we always obtain the high-spin state because of the large Hund's rule coupling of 3d transition metal ions. This will directly lead to the insulating state of Fe-doped samples, because the Fe^{3+} has five 3d electrons, favoring the $d^5 \uparrow d^0 \downarrow$ configuration in a high-spin state and resulting in a gap between the majority and minority spins. For the Cr-doped case, the local environment of dopants, which substitute the Bi sites, is an octahedron formed by six nearest neighboring Se^{2-} ions. Such a local crystal field splits the d shell into t_{2g} and e_g manifolds. This splitting is large enough to stabilize the $t_{2g}^3 e_g^0 \uparrow t_{2g}^0 e_g^0 \downarrow$ configuration of a Cr^{3+} ion, resulting in a gap between the t_{2g} and e_g manifolds. For the case of Ti or V doping, even the t_{2g} manifold is partially occupied, leading to the metallic state. We note that, although the local density approximation (LDA) in the density functional theory may underestimate the electron correlation effects, the inclusion of electron-electron interaction U (such as in the LDA + U method) should further enhance the gap [it may also reduce the p - d hybridization and T_c (27)].

The energy gain because of the spin polarization is about 0.9 eV per Fe, 1.5 eV per Cr, 0.7 eV per V and 0.02 eV per Ti, respectively, which are large numbers except for the case of Ti substitution. From the spin splitting of p orbitals of the band electrons, the estimated effective exchange coupling J_{eff} between the local moments and

the p electrons (28) is around 2.7 eV for Cr and 2.8 eV for Fe in Bi_2Se_3 , comparable to that in GaMnAs (28, 29). The exchange splitting introduced by the magnetic dopants can be estimated as $\Delta E = xJ_{\text{eff}}\langle S \rangle$, where x is the doping concentration and $\langle S \rangle$ is the mean field expectation value of the local spin. By using this value of J_{eff} and applying a mean field theory, we can estimate the FM Curie temperature to be of the order of tens of K, in the range accessible by experiments (24).

Once the FM order is achieved in TI, the QAH effect can be realized in 2D thin films of such systems. Because the bulk states are always gapped, we focus on the simplest low-energy effective Hamiltonian consisting of Dirac-type surface states only,

$$H_{\text{sf}} + H_{\text{Zeeman}} = \begin{bmatrix} 0 & iv_F k_- & m_k^* & 0 \\ -iv_F k_+ & 0 & 0 & m_k^* \\ m_k & 0 & 0 & -iv_F k_- \\ 0 & m_k & iv_F k_+ & 0 \end{bmatrix} + \begin{bmatrix} gM & 0 & 0 & 0 \\ 0 & -gM & 0 & 0 \\ 0 & 0 & gM & 0 \\ 0 & 0 & 0 & -gM \end{bmatrix} \quad (2)$$

with the basis of $|t\uparrow\rangle$, $|t\downarrow\rangle$, $|b\uparrow\rangle$, and $|b\downarrow\rangle$, where t , b represent the surface states sitting on the top and bottom surfaces and $\uparrow$, $\downarrow$ represent the spin up and down states, respectively. v_F is the Fermi velocity, m_k describes the tunneling effect between the top and bottom surface states, g is the effective g factor, $k_{\pm} = k_x \pm ik_y$, and M represents the exchange field along the z axis introduced by the FM ordering. For simplicity, spatial inversion symmetry is assumed, which requires that v_F , g , and M take the same values for top and bottom surfaces. In the thick slab geometry ($m_k \approx 0$), the spatially separated two pairs of surface states are well defined for the top and bottom surfaces. However, with the reduction of the film thickness, quantum tunneling between the top and bottom surfaces becomes more and more pronounced, giving rise to a finite mass term, m_k , which can be expanded up to the second order as $m_k = m_0 + B(k_x^2 + k_y^2)$, and the Hamiltonian can be rewritten in terms of the symmetric and antisymmetric combination of the surface states on top and bottom surfaces as

$$\tilde{H}_{\text{sf}} + \tilde{H}_{\text{Zeeman}} = \begin{bmatrix} m_k + gM & iv_F k_- & 0 & 0 \\ -iv_F k_+ & -m_k - gM & 0 & 0 \\ 0 & 0 & m_k - gM & -iv_F k_+ \\ 0 & 0 & iv_F k_- & -m_k + gM \end{bmatrix} = \begin{bmatrix} h_k + gM\sigma_z & 0 \\ 0 & h_k^* - gM\sigma_z \end{bmatrix} \quad (3)$$

with the following new basis: $|+\rangle$, $|-\rangle$, $|+\rangle$, $|-\rangle$, where $|\pm\rangle = (|t\uparrow\rangle \pm |b\uparrow\rangle)/\sqrt{2}$, $|\pm\rangle = (|t\downarrow\rangle \pm |b\downarrow\rangle)/\sqrt{2}$. Here, $h(k) = m_k\sigma_z + v_F(k_y\sigma_x - k_x\sigma_y)$,

similar to the Bernevig-Hughes-Zhang model describing the low-energy physics in a HgTe/CdTe quantum well (9). When $m_0B < 0$, band inversion occurs, and the system will be in the QSH phase if this is the only band inversion between two subbands with opposite parity. Regardless of whether this condition is satisfied, a strong enough exchange field will induce the QAH effect in this system, thanks to the presence of the σ_z matrix in the exchange field ($gM\sigma_z$) and the opposite signs of the Zeeman coupling terms in the upper and lower blocks of the effective Hamiltonian (Eq. 3). The exchange field increases the mass term of the upper block and reduces it for the lower block, breaking the time reversal symmetry. More importantly, a sufficiently large exchange field can change the Chern number of one of the two blocks. As illustrated in Fig. 3, if the four-band system is originally in the topologically trivial phase, the exchange field will induce a band inversion in the upper block and push the two subbands in the lower block even farther away from each other. Therefore, the 2D model with a negative mass in the upper block contributes e^2/h (where e is the charge of an electron and h is Planck's constant) for the Hall conductance. On the other hand, if the system is originally in the topologically nontrivial phase, both blocks have inverted band structures. In this case, a sufficiently large exchange field can increase the band inversion in the upper block and release it in the lower block. Again, the negative mass in the upper block contributes e^2/h for the Hall conductance. Such a mechanism is general for the thin-film TI systems with FM ordering; it is guaranteed by the fact that the surface states on the top and bottom surfaces have the same g factor. In HgMnTe , a further assumption that the electron and hole subbands have opposite signs of the exchange splitting (14) is required. This further justifies the robustness of the present proposal.

We carried out quantitative first-principles calculations for the Hall conductance in Bi_2Se_3 films, based on the Kubo formula (24). A spatial uniform exchange field is included to take into account the effect of magnetization in the FM state at the mean field level. In Fig. 4, A to C, we plot the lowest four subband levels at Γ point as a function of the exchange field. The level crossings between the lowest conduction bands (blue lines) and valence bands (red lines) are found for all three values of the layer thickness, signaling a quantum phase transition to the QAH state. In an insulator, where the chemical potential is located inside the energy gap between conduction and valence subbands, the Hall conductance is determined by the first Chern number of the occupied bands and must be an exact integer in the unit of e^2/h . The calculated Hall conductance for Bi_2Se_3 thin films with three typical thicknesses (Fig. 4D) has a jump from 0 to 1 at the corresponding critical exchange field for the level crossing.

Experimentally, the best way to see the QAH effect is to measure the Hall conductance as the function of gate voltage that tunes the chemical

potential. A quantized plateau in Hall conductance should be observed when the chemical potential is inside the gap (fig. S2). In real samples, a small amount of bulk carriers would always be present; however, if the concentration is low enough, they will be localized by disorder in two dimensions and will not affect the precise quantization of the Hall plateau.

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Does the Hydrated Electron Occupy a Cavity?

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Since the discovery of the hydrated electron more than 40 years ago, a general consensus has emerged that the hydrated electron occupies a quasispherical cavity in liquid water. We simulated the electronic structure and dynamics of the hydrated electron using a rigorously derived pseudopotential to treat the electron-water interaction, which incorporates attractive oxygen and repulsive hydrogen features that have not been included in previous pseudopotentials. What emerged was a hydrated electron that did not reside in a cavity but instead occupied a ~ 1 -nanometer-diameter region of enhanced water density. Both the calculated ground-state absorption spectrum and the excited-state spectral dynamics after simulated photoexcitation of this noncavity hydrated electron showed excellent agreement with experiment. The relaxation pathway involves a rapid internal conversion followed by slow ground-state cooling, the opposite of the mechanism implicated by simulations in which the hydrated electron occupies a cavity.

The nature of excess electrons in liquid water has been of continuing interest due to their important role in radiation chemistry and charge-transfer reactions. Excess electrons can be created directly by pulse radiolysis, or they can be formed after ionization of a solute if the detached electron resides in the liquid far from its parent cation. When liquid water locally contains one more electron than is needed to maintain electrical neutrality, the metastable localized species that is created has been termed the hydrated electron (e_{aq}^-). The hydrated electron has attracted considerable theoretical interest, in part because it poses the intriguing question of how a polar solvent acts to localize an object whose size and shape is determined self-consistently by interaction with its surroundings. Thus, although the hydrated electron is nominally a simple single-electron species, the many-body nature of its interactions with the surrounding water molecules has made this a nontrivial problem in statistical mechanics and quantum chemistry that can directly confront experiment.

Early continuum and semicontinuum models treated the hydrated electron as a spherical charge distribution with a radius determined self-consistently by polarization of the surrounding solvent. Such models provided insight into the mechanism by which electrons may be localized but did not give a microscopic picture for the structure of the solvent in the presence of the e_{aq}^- . Subsequent studies used molecular simulations to address such structural questions; in such simulations, the excess electron was treated quantum-mechanically, the surrounding water molecules were treated classically, and the electron-water interaction was described by what is known

formally as a pseudopotential (1–3). Although alternatives have been proposed (4), the consensus picture that emerged from such simulations was that a hydrated electron excludes water from a small region, so that the e_{aq}^- occupies a nearly spherical void that is surrounded by water molecules whose O–H bonds point toward the cavity (5). This picture is known as the cavity model of the hydrated electron, and because simulations in which the electron occupies a cavity agree reasonably well with experiment, the cavity model has come to dominate both experimental and theoretical discussions of the e_{aq}^- (6).

Despite this well-accepted view, important questions still remain about the properties of the hydrated electron. One ongoing controversy is the mechanism by which the e_{aq}^- relaxes to equilibrium after photoexcitation. The debate is whether relaxation occurs by rapid internal conversion to the ground state followed by slow reequilibration of the surrounding water molecules (7–9) or by slow internal conversion followed by rapid reequilibration on the ground state (10, 11). Another concern is that the cavity model predicts a strong anisotropy in polarized transient hole-burning experiments (12, 13), but this predicted signature is not seen experimentally (14, 15).

Here, we describe the results of molecular dynamics simulations based on a new, rigorously derived electron-water pseudopotential. The results of our calculations suggest that the hydrated electron does not occupy a cavity but instead encompasses a region of enhanced water density in which the electronic wavefunction overlaps ~ 37 water molecules. We calculated several distinct ground- and excited-state properties of this noncavity electron and, in every case, have found that our predictions are consistent with experiment. For certain properties, our simulations offer a better match to experiment than simulations in which the electron resides in a cavity. Thus, we suggest that previous claims that the hydrated electron occupies a cavity may have been premature and that a different physical picture of this important species may be more appropriate.

A key element in any simulation of the hydrated electron is the electron-water interaction specified by the pseudopotential. Although there is a formalism for deriving pseudopotentials using information from quantum chemistry calculations (16), the challenge lies in representing such pseudopotentials in a functional form that is convenient for molecular simulation. Thus, most bulk hydrated electron simulations have been based on ad hoc electron-water interactions (6, 17) or have used pseudopotentials that were constrained to have a simple functional form that neglected important details in the molecular-core region (2). Recently, however, we have shown that it is possible to generate rigorous molecular pseudopotentials directly from the orbitals obtained in quantum chemistry calculations (16). Thus, we decided to reexamine the properties of the hydrated electron using a rigorously derived electron-water pseudopotential (18), a two-dimensional contour plot of which is shown in the left panels of Fig. 1. Some of the notable features of our pseudopotential are the presence of deep attractive wells on each hydrogen atom, a substantial repulsion of the electron between the two hydrogen atoms, and

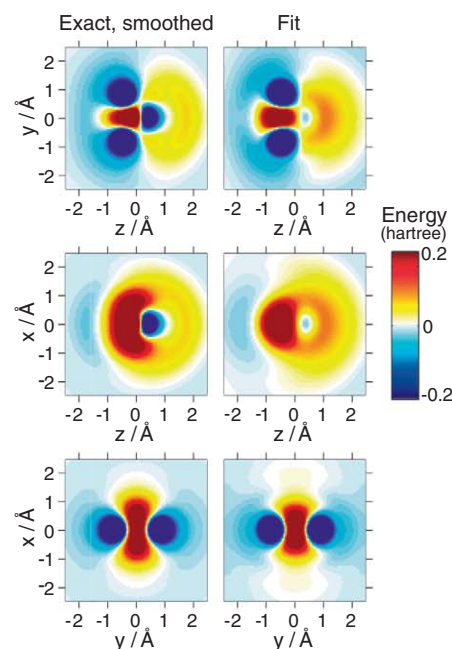


Fig. 1. Cuts of the full smoothed electron-water pseudopotential (left panels) and our fit to the full smoothed pseudopotential (right panels, eq. S5, and table S1); the energy scale is in Hartree. The water molecule lies in the y - z plane with the dipole pointing along the z axis. The O atom is at position $y = 0.0$, $z = 0.11663$ Å, and the H atoms are at $y = \pm 0.76001$, $z = -0.46654$ Å. The upper panels show a cut parallel to the molecular y - z plane with $x = 0.0260$ Å; the middle panels show a cut perpendicular to the plane of the molecule with $y = 0.0260$ Å (which is the closest plane to the principal axis of the molecule given our finite grid sampling); the bottom panels show a cut perpendicular to the molecular plane through the two H atoms.

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an attractive feature near the oxygen atom opposite the hydrogens. Although the presence of the latter two features has been noted in work examining water-cluster anions (3, 19), these features have not been incorporated into the pseudopotentials in previous bulk e_{aq}^- simulations (1, 2), so their importance to the behavior of the bulk hydrated electron has not been explored. Thus, to account for the presence of these features, we have fitted our potential to a sum of hydrogen- and oxygen-centered terms, plus a sum of terms centered midway between the two hydrogen atoms; the fit is shown in the right panels of Fig. 1, and the fitting parameters and functional form of our pseudopotential are given in table S1.

Using our rigorously determined potential, we have run extensive mixed quantum/classical molecular dynamics (MD) simulations of hundreds of water molecules confined with a single excess electron in a cubic box. The water-water interactions were governed by a flexible simple point charge model (18), and the simulation time step was 0.5 fs. The adiabatic eigenstates of the electron were found at every time step on a 32 by 32 by 32 cubic grid that was 18.17 Å on a side using the Lanczos algorithm in the manner described in (20); we verified that the eigenstates did not change by more than 0.03 eV when a 64^3 grid was used. The equilibrium (ground state) results displayed here come from 30 ps of dynamics with 499 water molecules in a box 24.64 Å on a side in the microcanonical ensemble (constant number of molecules, volume, and total energy) at an average temperature of ~300 K. The nonadiabatic, excited-state trajectories were performed with 200 water molecules in a box the same size as the cubic grid. A comparison of the spectroscopic and other properties found with 200 and 499 water molecules is given in figs. S3 and S4.

Figure 2 displays structural and electronic properties of the e_{aq}^- computed from these simulations. The black dashed and solid curves in Fig. 2A display average values of the hydrated electron's charge density and r^2 times this density, respectively, relative to the electron's average position, or center-of-mass (COM). These curves show that the hydrated electron is clearly not a point-particle but instead has charge distributed over a ~2.6 Å radius of gyration, in good agreement with the experimental value of ~2.5 Å obtained from spectral moment analysis (21). Fig. 2A also shows radial distribution functions (RDFs), which give the probability of finding either H atoms (red dashed curve) or O atoms (solid blue curve) on the surrounding water molecules relative to the electron's COM. Clearly, the electron's charge density has considerable overlap with regions containing a high density of water molecules: A major fraction of the charge density of the e_{aq}^- is found between the first hydrogen and oxygen peaks in the RDFs. This is seen explicitly in the snapshots shown in Fig. 2, B and C, which show that many water molecules reside inside the volume occupied by the hydrated electron.

The radial distribution functions (RDFs) in Fig. 2A make clear that the hydrated electron does not exclude a discrete volume about itself, so that a description of the e_{aq}^- as residing in a cavity is inappropriate. Although the RDFs become small at distances close to the electron's COM, the fall-off is gradual, more akin to the solvation structure around a soft sphere rather than a hard excluded volume. Moreover, the RDFs do not reach zero at the origin, indicating that solvent fluctuations occasionally allow water oxygen atoms to penetrate all the way to the hydrated electron's COM. The fact that there is a higher probability for H atoms to be close to the electron's COM than O atoms indicates that the electron induces a net orientational ordering of the water molecules inside the electron (an ordering that can also be seen in Fig. 2, B and C), although the preference for water O-H bonds to point toward the COM is reduced from that seen in cavity models of the electron (2, 5, 10).

The slight maxima and minima in the RDFs show that the electron also induces some translational structural organization of the nearby water molecules, but even the slight minimum in the oxygen RDF at ~3.5 Å lies above 1. Integration of the RDF gives an average number of molecules within 6 Å of the electron COM of ~37, whereas

only ~30 water molecules would be expected within this distance if the average local density were that of neat water. Thus, rather than pushing water away from the region of maximum electron density, as in the cavity model, our simulations show that the hydrated electron occupies a region of enhanced water density extending out ~6 Å. This result implies that the net interaction of the electron with water is attractive, inducing an electrostriction effect, in direct contrast to the overall repulsive interaction necessary to produce a cavity. The effect that the hydrated electron has on the structure of the nearby water molecules is summarized in Fig. 2D, which shows water-water RDFs for water molecules inside the electron (within 3.25 Å of the electron's COM, middle curves) and those in pure water at both normal (1.00 g/cm³, lower curves) and higher (1.23 g/cm³, upper curves) densities. Clearly, the water molecules inside the electron are packed together more like water at high density than like water at normal density.

Figure 3 shows various energetic and spectroscopic properties of the hydrated electron calculated with our potential. As expected, the time-dependent adiabatic energy levels shown in Fig. 3A fluctuate in response to water motions, but the energy levels differ in two ways from those calculated in simulations in which the elec-

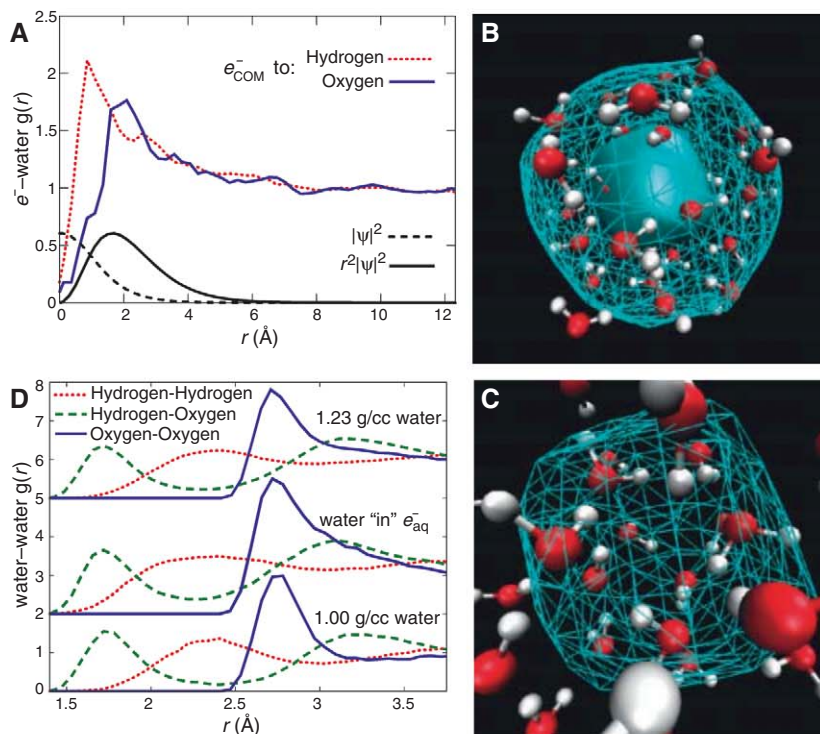


Fig. 2. (A) Electron COM-to-water site radial distribution functions (colored curves). The dashed curve is the square of the wavefunction as a function of distance from the COM, calculated as a time-average over 12.5 ps of dynamics, and the solid curve is this quantity multiplied by r^2 (both in arbitrary units). (B) All water molecules within 6.0 Å of the COM of the electron for a representative configuration. The wire mesh outer contour encloses 90% of the electron's charge density, and the opaque inner contour encloses 50%. (C) Expanded version of the configuration in (B), with the 50% contour represented by a mesh. (D) Water-water radial distribution functions for pure water at density 1.00 g/cm³ (lower curves), 1.23 g/cm³ (upper curves), and for water molecules located within 3.25 Å of the electron center of mass (middle curves). The three sets of curves have been shifted vertically for clarity.

tron resides in a cavity. First, in our calculations the excited states form a continuous band, unlike cavity models that have three low-lying excited states and then an energy gap below the higher-lying, so-called continuum states. Second, in our model, the solvent induces greater fluctuations in the ground-state energy than in the excited-state energies. In calculations with approximate pseudopotentials where the electron resides in a cavity, the fluctuations of the ground- and excited-state energies are of similar magnitude (1, 2, 10).

We calculated the optical absorption spectrum of the e_{aq}^- from our simulations by binning the energy gaps weighted by the square of the appropriate transition dipole moment for the lowest seven transitions from configurations taken every 10 fs (18), as shown by the red dotted curve in Fig. 3B. The agreement with the Gaussian-Lorentzian fit to the experimental spectrum (solid black curve) is remarkable: The spectrum calculated with our model matches the experimental spectrum more closely than calculations with pseudopotentials that lead to a cavity-based hydrated electron (1, 2, 10). Moreover, shifting the experimental spectrum 0.15 eV lower in energy (dashed black curve) shows that the shape of the low-energy edge of the calculated spectrum is correct. We do not expect to reproduce the high-energy tail of the spectrum in these calculations, both because of the small number of excited states we have included (calculations with 20 states on a few configurations show that the tail does begin to fill in) and because we have not accounted for quantum effects in the solvent (22). Fig. 3C shows a decomposition of the calculated absorption spectrum of the e_{aq}^- into sub-bands associated with transitions from the ground state to each of the lowest seven excited states. As in the cavity model, the bulk of the spectrum comes from transitions to the lowest three excited states. However, because there is no gap between the first three excited states and the higher-lying states, our computed spectrum does not show the unphysical dip between the bright states and the high-energy tail typically seen in spectra predicted by cavity models (1, 2, 6, 10, 17, 22).

The three lowest excited states that contribute the most to the absorption spectrum in our

model of the e_{aq}^- each have a positive and a negative lobe, as shown for a representative configuration in fig. S1, although the lobes are typically of different sizes. These three lowest-lying quasi- p -like states are substantially larger than the ground state of the hydrated electron, having average radii of gyration of 4.6(0.3), 5.0(0.5), and 5.4(0.6) Å, in increasing order of energy (root-mean-squared deviations given in parentheses). These radii match well with the estimates from excited-state scavenging experiments (23). Perhaps more interestingly, the three low-lying quasi- p -like states do not point in rigorously orthogonal directions: Dot products between the normalized transition dipoles from the ground to the three quasi- p -like states, $\cos \theta = \vec{u}_i \cdot \vec{u}_j$, are distributed roughly as $\exp(-\cos\theta/0.1)$, as shown in fig. S2. These deviations from strict orthogonality occur because small fluctuations in the positions of water molecules far from the electron COM can alter the orientation of one of the excited states without greatly affecting the other two. This is in sharp contrast to what occurs when the electron occupies a cavity: In that case, the cavity's shape strictly determines the relative orientations of the transition dipoles (supporting online text) (12, 24).

In the cavity model, the width of the electron's absorption spectrum reflects fluctuations in the shape of the cavity that change the splitting of three relatively well-separated sub-bands, representing transitions to the three bright p -like excited states (24). In contrast, our calculations suggest that the spectrum is broadened by large fluctuations in the ground-state energy, so the underlying individual absorption sub-bands overlap substantially and are not distinct, as shown in Fig. 3C. Each of the three bright sub-spectra spans roughly 3/4 of the width of the entire band, consistent with experimental estimates of the magnitude of homogeneous broadening in the hydrated electron spectrum (25). The fact that the transitions to the different excited states overlap in energy and have transition dipoles that are not strictly orthogonal suggests that there should be no detectable anisotropy in polarized pump-probe hole-burning measurements on the hydrated electron (15). This is a very different prediction

from the strong polarized anisotropy that emerges in simulations based on the cavity model (12, 13). Thus, unlike the cavity model, our calculations are consistent with the experimental observations that polarized hole-burning experiments on the e_{aq}^- show no anisotropy (14, 15).

In addition to the ground-state electronic structure, a sound model for the e_{aq}^- should also be able to correctly predict the relaxation dynamics after photoexcitation to one of the low-lying optically accessible excited states. Thus, we chose to compute the relaxation dynamics of a photoexcited hydrated electron with our model by running 20 nonadiabatic, nonequilibrium trajectories in which the electron is instantaneously resonantly excited by light of energy $1.55 \pm .01$ eV (~ 800 nm) (18). For probe wavelengths near the excitation wavelength, the red curve in Fig. 4C shows that the calculated transient dynamics of the e_{aq}^- involve bleach recovery on both fast (150 fs, 75% amplitude) and slow (1.1 ps, 25% amplitude) time scales (black curve). The transient dynamics in this region resemble the time traces of Barbara and co-workers (at a ~ 740 -nm pump wavelength, to account for the slight red shift of our calculated spectrum) (7). At wavelengths longer than the excitation pulse, our calculations predict an initial bleach followed by a delayed transient absorption (blue and green curves in Fig. 4C), also similar to the experimental data (11). We note that the magnitude of the delayed absorption should be slightly underestimated by our calculations because we compute transitions only to the lowest seven excited states. Figure 4D shows that this means that the agreement between our calculated transient absorption spectra and experiment is at least as good as—and possibly better than—that with the cavity model (dotted blue curve at $t = 1$ ps), which requires the calculated spectra to be red-shifted by nearly 0.7 eV to come close to matching the experimental data (dashed blue curve at $t = 1$ ps) (2, 12).

Simulations in which the electron resides in a cavity suggest that the transient absorption dynamics are governed primarily by solvation of the excited state, with a slow internal conversion followed by fast equilibration of the ground state

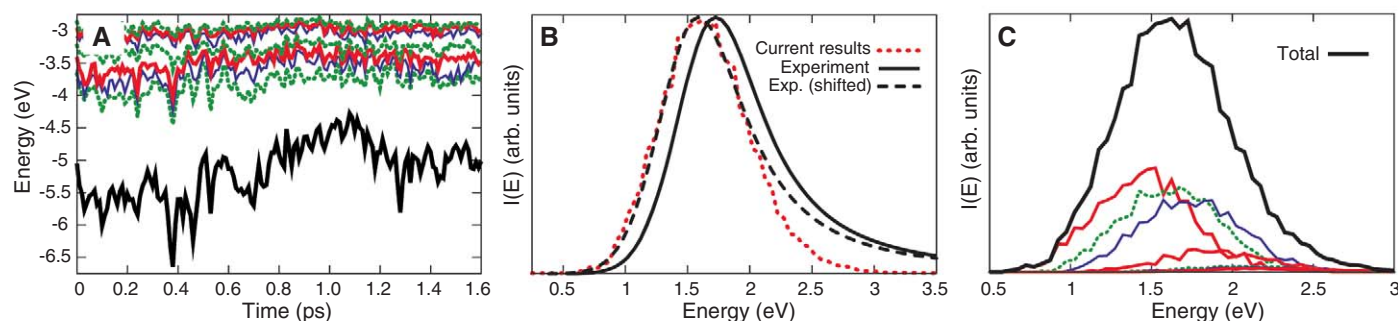


Fig. 3. Equilibrium properties of the hydrated electron. (A) Time evolution of the lowest eight adiabatic energy levels. (B) Calculated absorption spectrum (red dotted curve) from the ground to the lowest seven excited states, analytical fit to the experimental absorption spectrum [black solid

curve (30)], and experimental spectrum shifted by 0.15 eV (black dashed curve). (C) Total absorption spectrum (black curve) and absorption spectra associated with excitation to each of the lowest seven adiabatic states (colored curves).

(10, 12, 13). This adiabatic picture, however, is not the only way to interpret the observed spectral behavior. Early pump-probe measurements on the e_{aq}^- were explained in terms of a nonadiabatic model involving rapid internal conversion followed by slow cooling of a nonequilibrium ground state (7, 8). Moreover, more recent experiments studying the excitation of water cluster anions also strongly suggest that relaxation occurs by rapid internal conversion followed by slow ground-state cooling (9). The experimental literature on the relaxation of the bulk hydrated electron seems to be about evenly divided between the adiabatic and non-adiabatic relaxation mechanisms.

Figure 4A shows a representative excited-state trajectory of the hydrated electron simulated with our model. The salient features are that the ground-to-occupied energy gap closes very rapidly [an observation that is consistent

with photon echo experiments on the hydrated electron (26)]; the electron then makes a transition to the ground state rapidly, in ~ 280 fs; and after the transition, the ground-state energy returns to its equilibrium value slowly, in a time ≥ 1 ps. Figure 4B shows the probability of the hydrated electron remaining excited as a function of time after excitation for our 20-trajectory ensemble; the average excited-state lifetime is ~ 280 fs with a root-mean-squared deviation of ~ 150 fs. Taken together, our calculations predict that photoexcited hydrated electrons return to the ground state much faster than suggested by calculations in which the e_{aq}^- resides in a cavity (10, 20).

Because the underlying relaxation mechanism in our simulations is different, it is important to contrast our assignment of the delayed near-IR transient absorption to those from simulations in which the electron resides in a cavity. In our sim-

ulations, the induced absorption results from transitions originating from a nonequilibrated ground state of the e_{aq}^- , produced after rapid internal conversion. In the cavity model, the delayed absorption comes from so-called p -to- p transitions from the occupied first excited state to higher-lying excited states; both the energy gap and the transition dipole magnitudes of these transitions increase over the first few hundred fs (10). Thus, although both models appear to describe the pump-probe data roughly equally well, provided that an empirical shift of the predicted spectra is used for the cavity model, the interpretation of the dynamics underlying the spectral transients in the two models is completely different.

Finally, we note that our results also appear consistent with resonance Raman measurements on the hydrated electron (27), which showed that waters near the e_{aq}^- have both their bend and stretch frequencies red-shifted relative to those in pure water. Our model suggests that the water molecules that are strongly coupled to the electron are at an effectively higher density than water molecules in the bulk. It is well known that the Raman spectrum of high-density water under pressure shows down-shifts of both the bending and stretching modes, a result of the fact that O-H bonds are weakened when protons can be more equally shared between the O atoms of water molecules that are closer together (28). Thus, part of the major features seen in the Raman spectroscopy of the hydrated electron may result from the change in local water density and structure induced by the presence of the electron. In addition, the strong overlap of the electronic charge density with antibonding molecular orbitals on multiple water molecules also likely plays a role in weakening the OH bonds (25). The slight orientational ordering of the water molecules inside the electron is also consistent with the Raman spectroscopy, which shows that the two O-H bonds of the water molecules coupled to the electron experience different average environments (27).

How should recent *ab initio* simulations of the e_{aq}^- be viewed in light of our new model? Boero *et al.* have performed a DFT-based Car-Parrinello (CP) study of an excess electron amidst 32 water molecules, which predicted that the hydrated electron occupied a cavity (29), albeit one smaller than those seen in previous cavity-model calculations (1, 2, 10). The cavity in this CP simulation was transient, however, with a very short lifetime (29). We found that simulations with our model using smaller numbers of water molecules gave larger fluctuations in properties such as the electron's radius of gyration (supporting online text), so it is possible that with only 32 water molecules, the transient cavities Boero *et al.* observed were due to such fluctuations. Thus, we do not see any inconsistencies between our results and the CP simulations.

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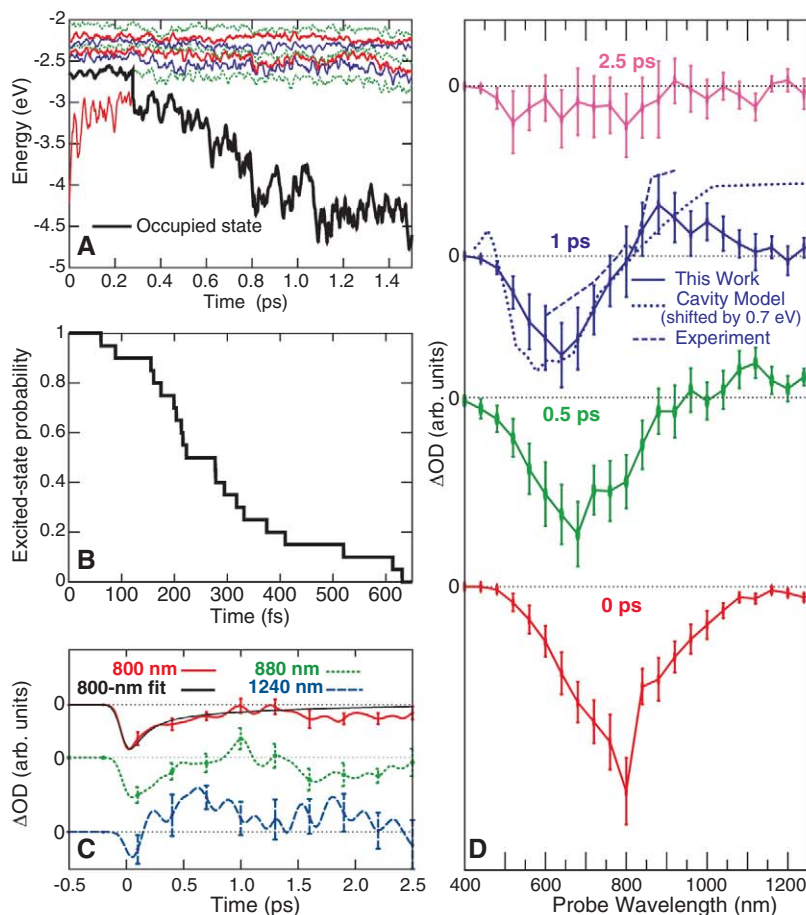


Fig. 4. Nonadiabatic excited-state relaxation dynamics of the hydrated electron. **(A)** Adiabatic energy levels (colored curves) as a function of time after excitation by 1.55 eV, with the heavy black curve indicating the occupied state. **(B)** The probability for the electron to remain in an excited state as a function of time after excitation for our 20 trajectories. **(C)** Calculated transient absorption dynamics at 130-fs time resolution for the wavelengths indicated; the curves have been offset vertically for clarity. The black curve shows a fit of our predicted results to a double-exponential decay with time scales of 150 fs and 1100 fs with a $\sim 3:1$ amplitude ratio, respectively, in excellent agreement with experimental data (7). **(D)** Simulated transient absorption spectra (solid curves) for the indicated times after excitation, with the curves offset vertically for clarity. Error bars are ± 1 SD of the mean. The dashed blue curve shows the experimental transient spectrum at 1 ps (11). The dotted blue curve shows the cavity model's simulated transient spectrum at 1 ps, shifted by -0.68 eV to aid comparison to the experiment (13).

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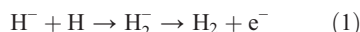
Experimental Results for H₂ Formation from H⁻ and H and Implications for First Star Formation

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During the epoch of first star formation, molecular hydrogen (H₂) generated via associative detachment (AD) of H⁻ and H is believed to have been the main coolant of primordial gas for temperatures below 10⁴ kelvin. The uncertainty in the cross section for this reaction has limited our understanding of protogalaxy formation during this epoch and of the characteristic masses and cooling times for the first stars. We report precise energy-resolved measurements of the AD reaction, made with the use of a specially constructed merged-beams apparatus. Our results agreed well with the most recent theoretically calculated cross section, which we then used in cosmological simulations to demonstrate how the reduced AD uncertainty improves constraints of the predicted masses for Population III stars.

More than 40 years ago, Saslaw and Zipoy (1) proposed that the formation of neutral molecular hydrogen played a central role in the cooling of primordial gas. Collisions of atomic H with molecular H₂ transfer kinetic energy from the H into internal energy of the H₂ through ro-vibrational excitation of the molecule. Subsequently, the molecule can radiatively relax and the emitted photon may escape from the cloud, thereby cooling the gas. Recent numerical models for the formation of the first (Population III) stars have demonstrated the importance of primordial H₂ as a coolant (2–4).

During the epoch of first star formation, H₂ was formed primarily via the associative detachment (AD) reaction (3),



Although this is the simplest anion-neutral chemical reaction, theory and experiment have failed to reach consensus for both the magnitude and energy dependence of the rate coefficient (5). This uncertainty severely limits our ability to model protogalaxies and metal-free stars forming from initially ionized gas, such as in H II regions created by earlier Population III stars (5, 6). For example, it limits our ability to predict whether a given protogalactic halo can cool and condense on a sufficiently short time scale before it is gravitationally disrupted through a collision with another protogalactic halo (5). Additionally, the cosmological simulations we have performed for this work show that for gas in free fall, the resulting spread in the minimum gas temperature—and in the gas density at which this minimum is reached—leads to a factor of >20 difference in the Jeans mass. Finally, for shocked gas under-

going isobaric collapse, the time required to reach the cosmic microwave background temperature can differ by up to a factor of 3. This affects the likelihood that such gas will cool and collapse to form a star before undergoing another shock (6).

The three most recent theoretical calculations for Eq. 1 have not converged, although they all used the same potential for the intermediate H₂⁻ anion. The results of Sakimoto (7) and Čížek *et al.* (8) are in good agreement with one another but not with the calculations of Launay *et al.* (9). The room-temperature flowing afterglow results of (10–12), however, are discrepant with the calculations of Sakimoto and Čížek *et al.* but are in reasonable agreement with those of Launay *et al.* See (13) for a brief review. Unfortunately, the existing experimental work provides no constraint on the temperature dependence for Eq. 1, which limits the ability of these measurements to benchmark theory. Considering all these issues, we have designed and built a dedicated merged-beams apparatus in order to perform energy-resolved AD measurements spanning the entire relevant collision energy range.

An overview of the experiment is shown in Fig. 1; a detailed description is given in (14). In the first leg of the apparatus, we extract an H⁻ beam from a negatively biased ion source to achieve a beam energy eU_s of ~10 keV. We collimate it via standard ion beam technology. The atomic H beam is generated in the central portion of the second leg of the apparatus, where ~7.4% of the H⁻ is neutralized by photodetachment (PD) with a laser diode array at 975 nm with continuous-wave power of 1.4 kW. At the photon energy (1.25 eV) and power density used, the PD process produces exclusively ground-state H atoms. The PD occurs in the center of a drift tube, 1.2 m in length, used to control the relative energy between the H⁻ and H beams. When a voltage $-U_f$ is applied to the tube, the H⁻ ions are decelerated as they enter the tube and are then accelerated

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back to their initial energy upon leaving. The neutral H atoms are created at an energy of $E_H = e(U_s - U_f)$ and maintain this energy after leaving the drift tube. Voltages of $-281 \text{ V} \leq -U_f \leq -1 \text{ V}$ were used, corresponding to center-of-mass collision energies E_{cm} from 3.7 meV to 1 eV. The lower end of the E_{cm} scale is limited by the angular spread of the merged beams (fig. S2), which are confined by two circular apertures before and after the PD region (5 mm in diameter, 2.8 m apart).

The interaction region begins with an electrostatic plate used to chop the H^- beam. The neutral beam is chopped by switching the laser. By chopping both beams out of phase with one another, the signal from H_2 generated in the interaction region can be extracted from the various backgrounds (14, 15). Two rotating-wire beam profile monitors are used to record the ion and neutral beam profiles needed to determine the merged-beams overlap form factor Ω (16, 17). The interaction region ends with an electrostatic quadrupole, which deflects the H^- ions into a Faraday cup used to continuously record the current. The neutral H particle current I_H (measured in amperes) in the interaction region is determined by calibrating the secondary electron emission of a neutral cup situated at the end of the beamline.

In the interaction region, the AD process creates $\sim 100 \text{ s}^{-1}$ of H_2 with a kinetic energy of 20 keV. The source potential is varied with U_f so that the sum of the parent H^- and H energies is 20 keV for all values of U_f (14). The H_2 is filtered from the $\sim 10 \text{ keV}$ stream of $\sim 4 \times 10^{11} \text{ s}^{-1}$ H atoms in two steps. First, the H_2 and H are sent through a differentially pumped gas cell (length $\sim 80 \text{ cm}$) with a He pressure of 2×10^{-4} torr. In the gas cell, electron stripping converts $\sim 5\%$ of the H_2 into H_2^+ . The stripping cross section for 20 keV H_2 in helium is $\sigma_{\text{st}} = 1.04 \times 10^{-16} \text{ cm}^2$ with an uncertainty of 16.5% (18). This constitutes the largest contribution to the uncertainty of our absolute scale. Experimental studies for stripping of room-temperature and ro-vibrationally excited H_2 on He and H_2 indicate that the unknown ro-vibrational distribution of the H_2 generated by the AD process introduces an additional 10% uncertainty (13, 19, 20).

The He column density (N_{He}) in the gas cell is determined by measuring the H^- loss from a traversing beam due to single and double electron detachment. The cross sections for these processes are well known (21, 22), allowing us to calibrate N_{He} to within 9% accuracy.

At the exit of the gas cell, two sequential electrostatic cylindrical energy analyzers select out the 20 keV H_2^+ ions and direct them onto a channel electron multiplier (CEM) with near-unity detection efficiency (23). After background subtraction, typical CEM signal rates S on the order of a few hertz are observed.

In our experiments, we measured the product of the AD cross section σ_{ad} and the relative velocity v_r convolved with the experimental velocity spread. The spread was calculated from geometric models of the overlapping beams, which showed excellent agreement with the beam shapes measured at the beam profile monitors. The measured rate coefficient is

$$\alpha = \langle \sigma_{\text{ad}} v_r \rangle = \frac{S}{\sigma_{\text{st}} N_{\text{He}}} \frac{e^2 v_{\text{H}^-} v_{\text{H}}}{I_{\text{H}^-} I_{\text{H}}} \frac{1}{\Omega} \quad (2)$$

where v_{H^-} and v_{H} are the velocities of the H^- and H beams, respectively, and the angle brackets denote the average over the velocity spread of the experiment. In Fig. 2, our experimental results are compared to the calculations of Čížek *et al.* (8). The agreement between experiment and theory in both magnitude and energy dependence is strong.

We have used the results of Čížek *et al.* to generate a thermal rate coefficient assuming a Maxwell-Boltzmann energy distribution. In (8) the cross section for Eq. 1 between 10^{-3} and 1 eV was calculated using a nonlocal resonance model in which the short-lived $\text{H}_2^{-2}\Sigma_u^+$ collision complex interacts with the $\text{H}_2 X^1\Sigma_g^+ + e^-$ continuum. To determine rate coefficients from 1 to 10^4 K , it was necessary for us to extend that work at lower energies (10^{-6} to 10^{-3} eV) and also at higher energies (1 to 10 eV), where almost 100 partial waves had to be included. The $\text{H}_2^{-2}\Sigma_g^+$ state was not taken into account because the repulsive character of this state suggests that the cross sections

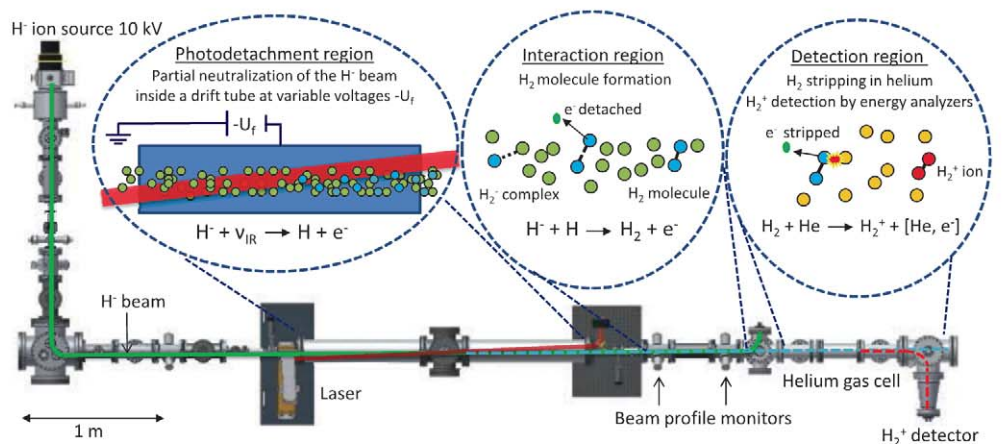
will be much smaller than contributions from the $\text{H}_2^{-2}\Sigma_u^+$ state. To simplify the implementation of our experimentally confirmed thermal rate coefficient into cosmological models, we provide an analytical fit function in table S1.

In gas that is cooling and recombining from an initially ionized state—for example, in an H II region surrounding a previous Population III star— H_2 forms efficiently via the AD reaction owing to the high abundance of free electrons. At times much greater than the first few recombination times, the H_2 fraction tends toward an almost constant value (24) that depends critically on the AD rate coefficient.

To examine the effect of our thermal AD rate coefficient on primordial star formation, we performed a set of high-resolution simulations of primordial gas evolving within an initially ionized protogalactic halo. The simulation is intended to represent a low-mass protogalaxy forming in a relic H II region. We represent the dark matter halo with a fixed background potential, assuming a Navarro-Frenk-White density profile (25) and a total dark matter mass of 9.7×10^5 solar masses ($M_\odot$). The associated gas mass of $2 \times 10^5 M_\odot$ has a starting temperature of 10^4 K and is initially in hydrostatic equilibrium. We simulate the evolution of the gas with a modified version of the GADGET 2 smoothed particle hydrodynamics (SPH) code (26) and represent the $2 \times 10^5 M_\odot$ of gas with 2 million SPH particles, giving us a particle mass of $0.1 M_\odot$ and a minimum mass resolution of $10 M_\odot$ (13).

Figure 3 compares the state of the gas for five different runs at the end of the simulations. Each point corresponds to a spherical shell of material centered about the cloud center (13). Runs 1 and 2 used the previous upper and lower limits for the rate coefficient of Eq. 1 from (5), respectively; run 3 used our new thermal rate coefficient, whereas runs 4 and 5 used values that were 25% larger or smaller than this, respectively, representing the remaining systematic uncertainty in the rate coefficient. The temperature for each run is plotted in Fig. 3A. In run 1, the high AD rate leads to a higher asymptotic H_2 abundance (Fig. 3B), and hence a higher HD abundance

Fig. 1. Schematic of the merged-beams apparatus used to measure the H_2 associative detachment reaction. Infrared laser photons are denoted by ν_{IR} .



(Fig. 3C), as HD is rapidly produced from H_2 by the reaction



In this run, the HD abundance becomes large enough to dominate the cooling. Because the critical density of HD—the density at which its rotational level populations approach their local thermodynamic equilibrium values—is much higher than that of H_2 , and because HD can cool the gas to much lower temperatures, the gas remains cold up to hydrogen nuclei number density $n \approx 10^6 \text{ cm}^{-3}$.

In run 2, the lower AD rate leads to a lower asymptotic H_2 abundance, and in this case the amount

of HD that forms is never sufficient to dominate the cooling. Therefore, the gas reaches a minimum temperature $T_{\min} \approx 200 \text{ K}$ at a density close to the H_2 critical density, and thereafter begins to heat up.

This difference in behavior has important consequences for the characteristic mass of the Population III stars that are formed, which is closely related to the Jeans mass of the gas at the density of minimum temperature (27). The Jeans mass is smaller in run 1 than in run 2 by a factor of >20 , demonstrating that the previous uncertainty in the AD rate coefficient results in a very large uncertainty in the predicted mass of the Population III stars.

Fig. 2. Experimental rate coefficient $\alpha = \langle \sigma_{ad} v_r \rangle$ for Eq. 1. The error bars show the 1σ statistical uncertainty of the measurement (13). The dashed error bands present the systematic experimental uncertainty of 25% (at an estimated 1σ level). The solid line depicts the theoretical results reported in (8) multiplied by v_r and convolved with the experimental velocity distribution, which is non-Maxwellian for collision energies above $\sim 0.01 \text{ eV}$. See fig. S3 for a plot of the thermal rate coefficient derived from the theoretical cross-section results. An analytical fit for the thermal rate coefficient is shown in table S1.

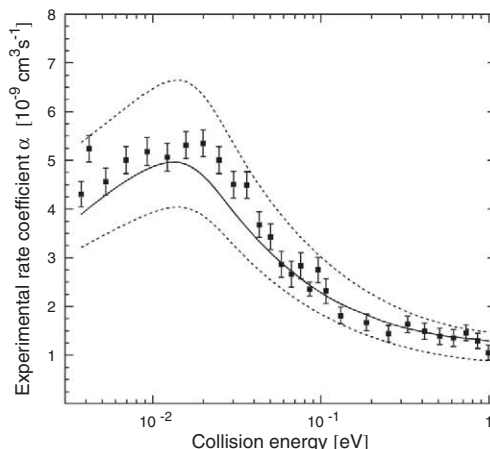
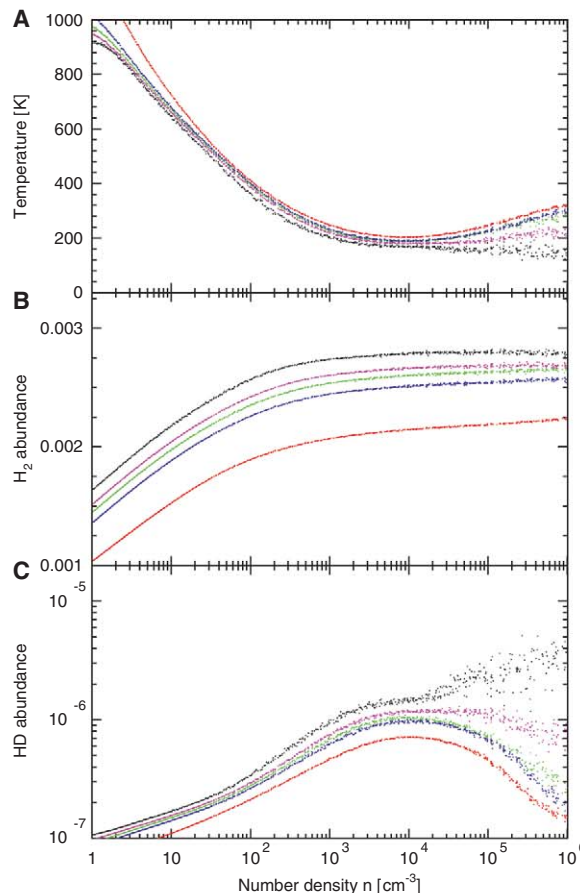


Fig. 3. (A to C) Simulations of primordial gas evolving in an initially ionized protogalactic halo, using different AD rate coefficients. Results are shown for (A) absolute temperature, (B) H_2 abundance, and (C) HD abundance. The black and red dots mark runs 1 and 2, representing the previous upper and lower limits for the AD rate coefficient, respectively. Run 3 (green) shows the results using our new thermal rate coefficient. For run 4 (magenta), a rate coefficient 25% greater than this was used, representing the remaining uncertainty in the AD rate; for run 5 (blue), a rate coefficient 25% smaller was used. The models were run at a redshift $z \approx 25$ where the cosmic microwave background temperature was $T_{\text{CMB}} \approx 70 \text{ K}$.



The results for runs 3 to 5 show that the measurements reported here explicitly constrain the situation. Runs 3 and 5 produce almost identical results, and although in run 4 slightly more HD is created and lower temperatures are reached, the qualitative evolution is the same as in the other runs. HD cooling never becomes dominant, and the uncertainty in the characteristic Population III mass is reduced from a factor of >20 to a factor of ~ 2 .

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Genetic Evidence for High-Altitude Adaptation in Tibet

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Tibetans have lived at very high altitudes for thousands of years, and they have a distinctive suite of physiological traits that enable them to tolerate environmental hypoxia. These phenotypes are clearly the result of adaptation to this environment, but their genetic basis remains unknown. We report genome-wide scans that reveal positive selection in several regions that contain genes whose products are likely involved in high-altitude adaptation. Positively selected haplotypes of *EGLN1* and *PPARA* were significantly associated with the decreased hemoglobin phenotype that is unique to this highland population. Identification of these genes provides support for previously hypothesized mechanisms of high-altitude adaptation and illuminates the complexity of hypoxia-response pathways in humans.

The Tibetan highlands are one of the most extreme environments inhabited by humans. Many present-day Tibetan populations are thought to be descendants of people who have occupied the Tibetan Plateau since the mid-Holocene, between 7000 and 5000 years ago (1), and possibly since the late Pleistocene, ~21,000 years ago (2, 3). Compared with Andean populations living in similar high-altitude conditions, Tibetans exhibit a distinct suite of physiologic traits: decreased arterial oxygen content, increased resting ventilation, lack of hypoxic pulmonary vasoconstriction, lower incidence of reduced birth weight, and reduced hemoglobin (Hb) concentration (on average, 3.6 g/dl less for both males and females) (4–8). Neighboring Han Chinese individuals and other nonadapted lowland visitors to high-altitude regions develop increased Hb concentration to compensate for the hypoxic high-altitude environment (9), and this response is associated with adverse effects (10, 11).

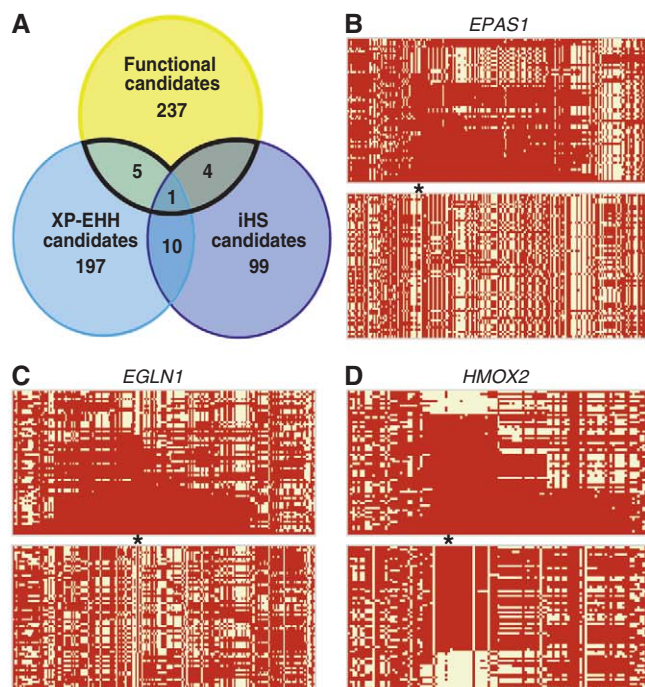
High-altitude Tibetans maintain normal aerobic metabolism, despite profound arterial hypoxia (4), perhaps through the existence of changes in the oxygen-transport system. For example, elevated circulating NO levels increase vasodilation and blood flow (12), which, when combined with increased ventilation (13), may increase the availability of oxygen to cells (4). Collectively, these traits strongly suggest that Tibetans have adapted uniquely to extreme high-altitude conditions. The genetic basis of this adaptation, however, remains unknown.

We used two intersecting criteria to identify genes potentially involved in high-altitude adaptation: First, a priori candidates for adaptation to high-altitude hypoxia were chosen because of their known functions (14). Second, a genome-wide scan was conducted to identify regions that show strong evidence of local positive selection in high-altitude Tibetans (Fig. 1). To generate a

set of a priori functional candidate loci, we constructed a list of Gene Ontology (GO) project categories (15) associated with the traits discussed above (Table 1). We merged genes from this list with those in the Panther-defined pathway “hypoxia response via activation of hypoxia-inducible factor (HIF)” (16), a major transcriptional regulator of oxygen homeostasis (17) that is probably associated with high-altitude adaptation. The resulting set of 247 functional candidate loci is listed in table S2.

We next identified alleles subject to strong recent positive selection (a selective sweep) in a sample of 31 unrelated Tibetans who were genotyped for one million single-nucleotide polymorphisms (SNPs) using the Affymetrix Genome-Wide Human SNP 6.0 Array. These individuals showed no evidence of admixture with neighboring populations [see supporting online material (SOM), figs. S1 and S2]. To pinpoint loci under positive selection, we first used the cross-population extended haplotype homozygosity (XP-EHH) statistic (18) to make comparisons between the Tibetan highland population and the combined HapMap Chinese (CHB) and Japanese (JPT) lowland populations (19). The XP-EHH statistic assesses haplotype differences between two populations and is designed to detect alleles that

Fig. 1. Gene regions responsible for adaptation to high-altitude hypoxia in Tibetans. **(A)** The strategy used to identify a list of genes related to high-altitude adaptation to hypoxia relies on three sets of genes. The set of functional candidates (yellow) consists of genes associated with physiological traits related to hypoxia (see Table 1 for categories). The XP-EHH (light blue) and iHS (dark blue) selection candidate sets include genes in the top 1% of the empirical distributions of XP-EHH and iHS results, respectively, excluding those with evidence of positive selection in neighboring populations (see SOM). The intersection of functional candidates with selection candidates (outlined in black) is enriched for regions containing genes that contribute to local adaptation to hypoxia in Tibetans. The genes in the intersection of functional candidates with iHS selection candidates still exhibit genetic variability in the population. **(B to D)** Comparison between Tibetan and CHB-JPT genomic regions identified in selection scans. The top and bottom halves of each figure represent chromosome regions in the Tibetan (number of chromosomes = 62) and CHB-JPT populations (62 randomly drawn chromosomes from 90 individuals), respectively, for the (B) *EPAS1*, (C) *EGLN1*, and (D) *HMOX2* genes identified in XP-EHH, both scans, and iHS, respectively. The three SNPs with the highest iHS and XP-EHH scores (indicated by an asterisk) were designated as the core haplotype for each genomic region. All haplotypes were sorted to the horizontal midline of each panel based on the length of uninterrupted matches to the reference sequence. See fig. S3 and table S5 for the remaining seven regions and details about these regions.



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The genes in the intersection of functional candidates with iHS selection candidates still exhibit genetic variability in the population. **(B to D)** Comparison between Tibetan and CHB-JPT genomic regions identified in selection scans. The top and bottom halves of each figure represent chromosome regions in the Tibetan (number of chromosomes = 62) and CHB-JPT populations (62 randomly drawn chromosomes from 90 individuals), respectively, for the (B) *EPAS1*, (C) *EGLN1*, and (D) *HMOX2* genes identified in XP-EHH, both scans, and iHS, respectively. The three SNPs with the highest iHS and XP-EHH scores (indicated by an asterisk) were designated as the core haplotype for each genomic region. All haplotypes were sorted to the horizontal midline of each panel based on the length of uninterrupted matches to the reference sequence. See fig. S3 and table S5 for the remaining seven regions and details about these regions.

have increased in frequency to the point of fixation or near-fixation in one of the populations (18, 19). A comparison with the CHB-JPT sample is appropriate because these populations have historically lived at low altitude and exhibit a small overall genetic distance to our Tibetan samples (figs. S1 and S2). We also identified partial selective sweeps (in which the adaptive variant is not yet near fixation) using the in-

tegrated haplotype score (iHS), a statistic based on the extent of decay of linkage disequilibrium surrounding a variant subjected to natural selection (20). The XP-EHH and iHS tests both have substantial statistical power to detect natural selection using genome-wide SNP genotypes, even when sample sizes are limited (19, 21).

Many selective events are likely to be shared among multiple populations, but we wish to focus only on those that are specific to high-altitude Tibetan populations. To do this, we divided the genome into consecutive, nonoverlapping 200-kb regions and excluded those that yielded significant values ($P < 0.01$) for the iHS test in neighboring Asian populations (see SOM). Seven of the 247 functional candidate loci were located in these excluded regions and were therefore eliminated from subsequent analyses. In our Tibetan sample, we then calculated an XP-EHH and iHS summary statistic (see SOM) for each genomic region, using an empirical significance level of 0.01 to identify a set of regions that exhibit evidence of local positive selection.

This set of regions contained 10 of the 240 genes from our functional candidate list (Fig. 1 and Table 2). Six of these genes were identified by the XP-EHH test, and five were identified by the iHS test. *EGLN1* was identified by both, and a previous study suggests that this gene may also be

under selection in Andeans (22). *EGLN1* and *EPAS1* are both in the HIF pathway (23) and show elevated F_{ST} values (fig. S4). Three additional loci, *EDNRA*, *PTEN*, and *PPARA*, are also associated with HIF activity (Fig. 2) (24, 25). *PPARA* and *ANGPTL4* are in the peroxisome proliferator-activated receptor (PPAR) lipid metabolism pathway, and *CYP17A1* and *CYP2E1* are cytochrome P450 genes (23). The two remaining genes are *HMOX2*, involved in HIF-independent oxygen sensing (26) and hypoxic endothelial cell survival (27), and *CAMK2D*, which mediates NO production in response to changes in intracellular calcium (28). Because the selection signals correspond to 200-kb sections of the genome rather than to individual genes, we performed additional analyses to localize the selection signal within each of the 10 regions (29). For each gene of interest, we observed multiple localization signals near the gene or bracketing the gene (fig. S4).

These results suggest that high-altitude adaptation in Tibetans has resulted from local positive selection on several distinct genes. However, even in the absence of selection, some loci will appear in the intersection of our functional candidate list and selection screens just by chance. To test the statistical significance of the observed pattern, we carried out a randomization test by resampling sets of 240 loci 1 million times from a list of all known autosomal genes (30). For each set of randomly chosen loci, we tabulated the number of loci that intersected the genomic regions identified by our selection scans. On average, the XP-EHH and

Table 1. Categories used to define the functional candidate a priori gene list. *N*, number of genes in each category. Total number of unique genes: 247.

Source	Accession	<i>N</i>
<i>Gene Ontology (GO) categories</i>		
Detection of oxygen	GO:0003032	14
NO metabolic process	GO:0046209	37
Oxygen sensor activity	GO:0019826	2
Oxygen binding	GO:0019825	46
Oxygen transport	GO:0015671	29
Oxygen transporter activity	GO:0005344	14
Response to hypoxia	GO:0001666	141
Response to oxygen levels	GO:0070482	157
Vasodilation	GO:0042311	41
<i>Panther pathway</i>		
Hypoxia response via HIF activation	P00030	33

Table 2. List of putatively advantageous genes identified by the intersection of functional and selection candidate gene lists. An en dash (–) indicates nonsignificant *P* values (see tables S11 and S12 for all values). All gene descriptions are based on RefSeq (23) unless otherwise noted.

Gene identified in 200-kb region	XP-EHH	XP-EHH empirical uncorrected <i>P</i> value	iHS	iHS empirical uncorrected <i>P</i> value
<i>EPAS1</i> Endothelial PAS domain protein 1	0.82	0.002	–	–
<i>CYP2E1</i> Cytochrome P450, family 2, subfamily E, polypeptide 1	0.71	0.007	–	–
<i>EDNRA</i> Endothelin receptor type A	0.7	0.008	–	–
<i>ANGPTL4</i> Angiopoietin-like 4	0.69	0.008	–	–
<i>CAMK2D</i> Calcium/calmodulin-dependent protein kinase II delta	0.68	0.009	–	–
<i>EGLN1</i> Egl nine homolog 1	0.96†, 0.86†*	0.0002†, 0.001†*	2.68*	0.01*
<i>HMOX2</i> Heme oxygenase (decycling) 2	–	–	3	0.001
<i>CYP17A1</i> Cytochrome P450, family 17, subfamily A, polypeptide 1	–	–	4	0.007
<i>PPARA</i> Peroxisome proliferator-activated receptor alpha	–	–	3.58	0.009
<i>PTEN</i> Phosphatase and tensin homolog	–	–	3.9	0.007

**EGLN1* was significant for both iHS and XP-EHH analyses.

†*EGLN1* spanned two genomic regions for XP-EHH.

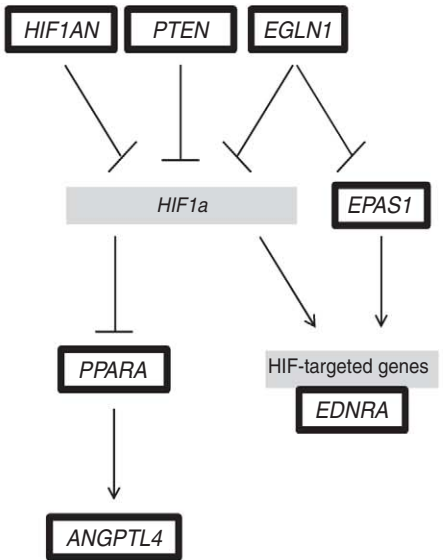


Fig. 2. Selection candidates involved in the HIF pathway. Genes identified as selection candidates that are related to the HIF pathway (outlined in black boxes) are illustrated below. [Gene descriptions and regulation during hypoxic/normoxic conditions are provided in table S2. Note that *HIF1AN* was not included on the a priori functional candidate list but was identified by both XP-EHH and iHS analyses (see SOM, tables S2, S11, and S12).] Genes indicated in the gray boxes are provided for reference.

iHS intersections contained 2.7 and 1.4 genes, respectively, significantly fewer than the six ($P < 0.05$) and five ($P < 0.01$) genes actually observed.

In contrast to the fixed or nearly fixed alleles detected by XP-EHH, the iHS test identifies regions affected by incomplete selective sweeps. Therefore, inter-individual genetic variation should be observable at these loci, allowing us to test for their association with Hb concentration, which is highly heritable and has distinctively low levels in high-altitude Tibetan populations (4). In each of the five 200-kb regions identified as targets of selection by iHS, we defined the selected core haplotype as the one containing the three SNP alleles that exhibit the most extreme iHS value (see SOM, table S6). We then used stepwise linear regression to test for correlations between Hb concentration and these five core haplotypes. The five independent predictor variables are the numbers of putatively advantageous haplotypes (0, 1, or 2) present in each 200-kb region. Because sex and age affect Hb concentration in Tibetans and Han Chinese, and the age effect differs between males and females (9), covariates are included in the regression analysis to allow an independent age effect in males and females (see SOM, tables S7 to S9).

The putatively advantageous haplotypes of *EGLN1* and *PPARA* both show significant negative correlations with Hb concentration ($P < 0.002$ and 0.0009 , respectively) (tables S8 and S9). A genome-wide regression analysis showed no excess of associations (398,020 SNPs with minor allele frequency ≥ 0.15 and no missing genotypes) (see SOM, figs. S1, S2, and S5). The phenotypic effects are substantial: Each additional copy of an advantageous haplotype at either

locus decreases Hb concentration by ~ 1.7 g/dl on average (Fig. 3). This effect is greater than the well-established sex-related difference in high-altitude Tibetans (~ 1.1 g/dl) (9). The strong and significant association between Hb concentration and haplotype variation at *EGLN1* and *PPARA* provides evidence of a genetic contribution to a form of high-altitude adaptation that appears to be unique to Tibetan populations.

A potential explanation for the correlation between *EGLN1* and decreased Hb concentration lies in the regulation of HIF and its target genes. *EGLN1* targets two HIF α proteins for degradation under normoxic conditions, decreasing the transcription of HIF-regulated targets such as *EPO*, the erythropoietin gene whose product induces red blood cell (RBC) production (Fig. 2). Furthermore, mutations in *EGLN1* prevent targeted degradation of HIF, leading to polycythemia (excessive production of RBCs) in mice and humans (31).

Although *PPARA* has not previously been considered as a candidate gene for high-altitude adaptation, it interacts with components of the HIF pathway. *PPARA* expression is inhibited by HIF1 during hypoxia in mice (Fig. 2) (25), and genes targeted by HIF are regulated by a HIF-independent mechanism involving PPARG coactivator-1 α (32). In addition, a *PPARA* agonist, the antidiabetic agent tesaglitazar, resulted in decreased Hb levels during human clinical trials (33). This effect is consistent with the correlation between the putatively advantageous *PPARA* haplotype and Hb concentration found here.

It is plausible that the diminished Hb levels found in Tibetans offset complications associated with sustained high Hb levels (for instance,

hyperviscosity) seen in non-Tibetans exposed to high-altitude conditions (10, 11). Alternatively, decreased Hb levels could be a side effect of other phenotypes that are the actual targets of natural selection. Functional analysis of genes such as *EGLN1* and *PPARA*, which have undergone positive selection and are associated with Hb levels in this Tibetan sample, will further increase our understanding of genetic adaptation to high-altitude environments. In addition, we will better understand the human response to hypoxia, which has important implications for the prevention and treatment of mountain sickness, high-altitude pulmonary and cerebral edema, and other hypoxia-related diseases.

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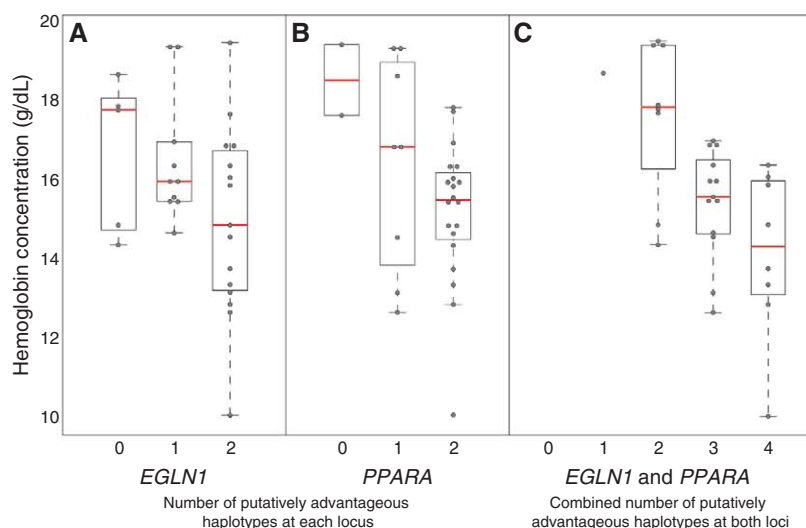


Fig. 3. Genotype-phenotype association of the inferred adaptive *EGLN1* and *PPARA* haplotypes with Hb concentration. Hb concentrations are plotted against the number of putatively advantageous haplotypes at (A) *EGLN1* and (B) *PPARA* (from 0 to 2 haplotype copies) for 30 Tibetan individuals. A box-and-whisker plot is overlaid on the data points to show the median (red line), upper and lower quartiles (box ends), and most extreme measurements (whiskers). (C) Because the effects at the two loci are similar in magnitude, direction, and significance, they are combined here for purposes of illustration. Hb concentrations are plotted against the combined number of *EGLN1* and *PPARA* haplotypes carried by an individual for both loci (from 0 to 4 copies) (table S9).

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Omnibus, with accession code GSE21661. These data, as well as phenotype data, are also available on our laboratory Web site, <http://jorde-lab.genetics.utah.edu>. Please contact R.L.G. for access to DNA samples.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1189406/DC1
Materials and Methods

Figs. S1 to S6
Tables S1 to S12
References

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Sequencing of 50 Human Exomes Reveals Adaptation to High Altitude

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Residents of the Tibetan Plateau show heritable adaptations to extreme altitude. We sequenced 50 exomes of ethnic Tibetans, encompassing coding sequences of 92% of human genes, with an average coverage of 18× per individual. Genes showing population-specific allele frequency changes, which represent strong candidates for altitude adaptation, were identified. The strongest signal of natural selection came from endothelial Per-Arnt-Sim (PAS) domain protein 1 (*EPAS1*), a transcription factor involved in response to hypoxia. One single-nucleotide polymorphism (SNP) at *EPAS1* shows a 78% frequency difference between Tibetan and Han samples, representing the fastest allele frequency change observed at any human gene to date. This SNP's association with erythrocyte abundance supports the role of *EPAS1* in adaptation to hypoxia. Thus, a population genomic survey has revealed a functionally important locus in genetic adaptation to high altitude.

The expansion of humans into a vast range of environments may have involved both cultural and genetic adaptation. Among the most severe environmental challenges to confront human populations is the low oxygen availability of high-altitude regions such as the Tibetan Plateau. Many residents of this region

live at elevations exceeding 4000 m, experiencing oxygen concentrations that are about 40% lower than those at sea level. Ethnic Tibetans possess heritable adaptations to their hypoxic environment, as indicated by birth weight (1), hemoglobin levels (2), and oxygen saturation of blood in infants (3) and adults after exercise (4). These results imply a history of natural selection for altitude adaptation, which may be detectable from a scan of genetic diversity across the genome.

We sequenced the exomes of 50 unrelated individuals from two villages in the Tibet Autonomous Region of China, both at least 4300 m in altitude (5). Exonic sequences were enriched with the NimbleGen (Madison, WI) 2.1M exon capture array (6), targeting 34 Mb of sequence from exons and flanking regions in nearly 20,000 genes. Sequencing was performed with the Illumina (San Diego, CA) Genome Analyzer II platform, and reads were aligned by using SOAP (7) to the reference human genome [National Center for Biotechnology Information (NCBI) Build 36.3].

Exomes were sequenced to a mean depth of 18× (table S1), which does not guarantee confident inference of individual genotypes. Therefore, we statistically estimated the probability of each possible genotype with a Bayesian algorithm (5) that

also estimated single-nucleotide polymorphism (SNP) probabilities and population allele frequencies for each site. A total of 151,825 SNPs were inferred to have >50% probability of being variable within the Tibetan sample, and 101,668 had >99% SNP probability (table S2). Sanger sequencing validated 53 of 56 SNPs that had at least 95% SNP probability and minor allele frequencies between 3% and 50%. Allele frequency estimates showed an excess of low-frequency variants (fig. S1), particularly for nonsynonymous SNPs.

The exome data was compared with 40 genomes from ethnic Han individuals from Beijing [the HapMap CHB sample, part of the 1000 genomes project (<http://1000genomes.org>)], sequenced to about fourfold coverage per individual. Beijing's altitude is less than 50 m above sea level, and nearly all Han come from altitudes below 2000 m. The Han sample represents an appropriate comparison for the Tibetan sample on the basis of low genetic differentiation between these samples ($F_{ST} = 0.026$). The two Tibetan villages show minimal evidence of genetic structure ($F_{ST} = 0.014$), and we therefore treated them as one population for most analyses. We observed a strong covariance between Han and Tibetan allele frequencies (Fig. 1) but with an excess of SNPs at low frequency in the Han and moderate frequency in the Tibetans.

Population historical models were estimated (8) from the two-dimensional frequency spectrum of synonymous sites in the two populations. The best-fitting model suggested that the Tibetan and Han populations diverged 2750 years ago, with the Han population growing from a small initial size and the Tibetan population contracting from a large initial size (fig. S2). Migration was inferred from the Tibetan to the Han sample, with recent admixture in the opposite direction.

Genes with strong frequency differences between populations are potential targets of natural selection. However, a simple ranking of F_{ST} values would not reveal which population was affected by selection. Therefore, we estimated population-specific allele frequency change by including a third, more distantly related population. We thus examined exome sequences from 200 Danish individuals, collected and analyzed as described for the Tibetan sample. By comparing the three pairwise F_{ST} values between these three samples, we can estimate the frequency change that occurred in the Tibetan population since its divergence from the Han population (5, 9). We found that this population branch statistic (PBS) has strong power to detect recent natural selection (fig. S3).

Genes showing extreme Tibetan PBS values represent strong candidates for the genetic basis

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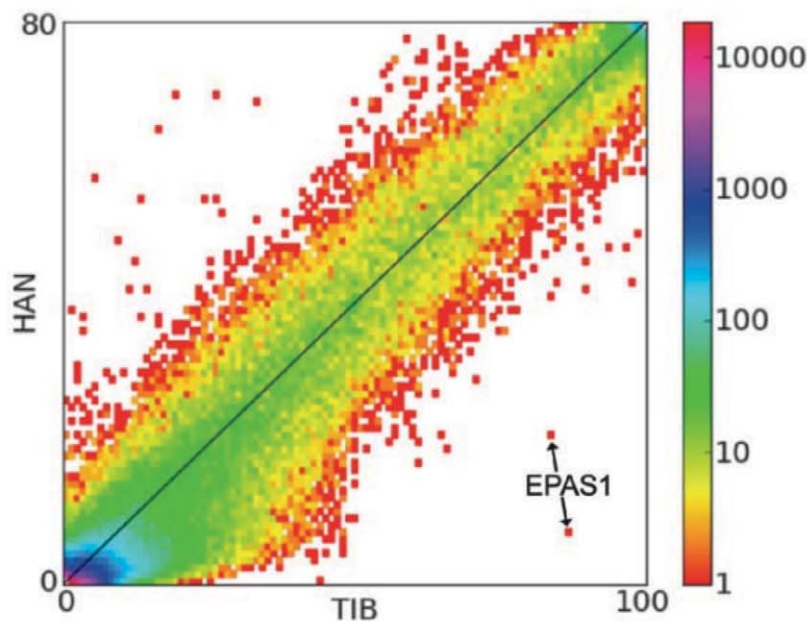


Fig. 1. Two-dimensional unfolded site frequency spectrum for SNPs in Tibetan (x axis) and Han (y axis) population samples. The number of SNPs detected is color-coded according to the logarithmic scale plotted on the right. Arrows indicate a pair of intronic SNPs from the *EPAS1* gene that show strongly elevated derived allele frequencies in the Tibetan sample compared with the Han sample.

Table 1. Genes with strongest frequency changes in the Tibetan population. The top 30 PBS values for the Tibetan branch are listed. Oxygen-related candidate genes within 100 kb of these loci are noted. For FXYD, F indicates Phe; Y, Tyr; D, Asp; and X, any amino acid.

Gene	Description	Nearby candidate	PBS	P value
<i>EPAS1</i>	Endothelial PAS domain protein 1 (HIF-2α)	(Self)	0.514	<0.000001
<i>C1orf124</i>	Hypothetical protein LOC83932	<i>EGLN1</i>	0.277	0.000203
<i>DISC1</i>	Disrupted in schizophrenia 1	<i>EGLN1</i>	0.251	0.000219
<i>ATP6V1E2</i>	Adenosine triphosphatase (ATPase), H+ transporting, lysosomal 31 kD, V1	<i>EPAS1</i>	0.246	0.000705
<i>SPP1</i>	Secreted phosphoprotein 1		0.238	0.000562
<i>PKLR</i>	Pyruvate kinase, liver, and RBC	(Self)	0.230	0.000896
<i>C4orf7</i>	Chromosome 4 open reading frame 7		0.227	0.001098
<i>PSME2</i>	Proteasome activator subunit 2		0.222	0.001103
<i>OR10X1</i>	Olfactory receptor, family 10, subfamily X	<i>SPTA1</i>	0.218	0.000950
<i>FAM9C</i>	Family with sequence similarity 9, member C	<i>TMSB4X</i>	0.216	0.001389
<i>LRRC3B</i>	Leucine-rich repeat-containing 3B		0.215	0.001405
<i>KRTAP21-2</i>	Keratin-associated protein 21-2		0.213	0.001470
<i>HIST1H2BE</i>	Histone cluster 1, H2be	<i>HFE</i>	0.212	0.001568
<i>TTL3</i>	Tubulin tyrosine ligase-like family, member 3		0.206	0.001146
<i>HIST1H4B</i>	Histone cluster 1, H4b	<i>HFE</i>	0.204	0.001404
<i>ACVR1B</i>	Activin A type IB receptor isoform a precursor	<i>ACVRL1</i>	0.198	0.002041
<i>FXYD6</i>	FXYD domain-containing ion transport regulator		0.192	0.002459
<i>NAGLU</i>	Alpha-N-acetylglucosaminidase precursor		0.186	0.002834
<i>MDH1B</i>	Malate dehydrogenase 1B, nicotinamide adenine dinucleotide (NAD) (soluble)		0.184	0.002113
<i>OR6Y1</i>	Olfactory receptor, family 6, subfamily Y	<i>SPTA1</i>	0.183	0.002835
<i>HBB</i>	Beta globin	(Self), <i>HBG2</i>	0.182	0.003128
<i>OTX1</i>	Orthodenticle homeobox 1		0.181	0.003235
<i>MBNL1</i>	Muscleblind-like 1		0.179	0.002410
<i>IFI27L1</i>	Interferon, alpha-inducible protein 27-like 1		0.179	0.003064
<i>C18orf55</i>	Hypothetical protein LOC29090		0.178	0.002271
<i>RFX3</i>	Regulatory factor X3		0.176	0.002632
<i>HBG2</i>	G-gamma globin	(Self), <i>HBB</i>	0.170	0.004147
<i>FANCA</i>	Fanconi anemia, complementation group A	(Self)	0.169	0.000995
<i>HIST1H3C</i>	Histone cluster 1, H3c	<i>HFE</i>	0.168	0.004287
<i>TMEM206</i>	Transmembrane protein 206		0.166	0.004537

of altitude adaptation. The strongest such signals include several genes with known roles in oxygen transport and regulation (Table 1 and table S3). Overall, the 34 genes in our data set that fell under the gene ontology category “response to hypoxia” had significantly greater PBS values than the genome-wide average ($P = 0.00796$).

The strongest signal of selection came from the endothelial *Per-Arnt-Sim (PAS) domain protein 1 (EPAS1)* gene. On the basis of frequency differences among the Danes, Han, and Tibetans, *EPAS1* was inferred to have a very long Tibetan branch relative to other genes in the genome (Fig. 2). In order to confirm the action of natural selection, PBS values were compared against neutral simulations under our estimated demographic model. None of one million simulations surpassed the PBS value observed for *EPAS1*, and this result remained statistically significant after accounting for the number of genes tested ($P < 0.02$ after Bonferroni correction). Many other genes had uncorrected P values below 0.005 (Table 1), and, although none of these were statistically significant after correcting for multiple tests, the functional enrichment suggests that some of these genes may also contribute to altitude adaptation.

EPAS1 is also known as *hypoxia-inducible factor 2α (HIF-2α)*. The HIF family of transcription factors consist of two subunits, with three

alternate α subunits (*HIF-1 α* , *HIF-2 α* /*EPAS1*, *HIF-3 α*) that dimerize with a β subunit encoded by *ARNT* or *ARNT2*. *HIF-1 α* and *EPAS1* each act on a unique set of regulatory targets (10), and the narrower expression profile of *EPAS1* includes adult and fetal lung, placenta, and vascular endothelial cells (11). A protein-stabilizing mutation in *EPAS1* is associated with erythrocytosis (12), suggesting a link between *EPAS1* and the regulation of red blood cell production.

Although our sequencing primarily targeted exons, some flanking intronic and untranslated region (UTR) sequence was included. The *EPAS1* SNP with the greatest Tibetan-Han frequency difference was intronic (with a derived allele at 9% frequency in the Han and 87% in the Tibetan sample; table S4), whereas no amino acid-changing variant had a population frequency difference of greater than 6%. Selection may have acted directly on this variant, or another linked noncoding variant, to influence the regulation of *EPAS1*. Detailed molecular studies will be needed to investigate the direction and the magnitude of gene expression changes associated with this SNP, the tissues and developmental time points affected, and the downstream target genes that show altered regulation.

Associations between SNPs at *EPAS1* and athletic performance have been demonstrated (13). Our data set contains a different set of SNPs, and we conducted association testing on the SNP with the most extreme frequency difference, located just upstream of the sixth exon. Alleles at this SNP tested for association with blood-related phenotypes showed no relationship with oxygen saturation. However, significant associations were discovered for erythrocyte count (F test $P = 0.00141$) and for hemoglobin concentration (F test $P = 0.00131$), with significant or marginally significant P values for both traits when each

village was tested separately (table S5). Comparison of the *EPAS1* SNP to genotype data from 48 unlinked SNPs confirmed that its P value is a strong outlier (5) (fig. S4).

The allele at high frequency in the Tibetan sample was associated with lower erythrocyte quantities and correspondingly lower hemoglobin levels (table S4). Because elevated erythrocyte production is a common response to hypoxic stress, it may be that carriers of the “Tibetan” allele of *EPAS1* are able to maintain sufficient oxygenation of tissues at high altitude without the need for increased erythrocyte levels. Thus, the hematological differences observed here may not represent the phenotypic target of selection and could instead reflect a side effect of *EPAS1*-mediated adaptation to hypoxic conditions. Although the precise physiological mechanism remains to be discovered, our results suggest that the allele targeted by selection is likely to confer a functionally relevant adaptation to the hypoxic environment of high altitude.

We also identified components of adult and fetal hemoglobin (*HBB* and *HBB2*, respectively) as putatively under selection. These genes are located only ~20 kb apart (fig. S5), so their PBS values could reflect a single adaptive event. For both genes, the SNP with the strongest Tibetan-Han frequency difference is intronic. Although altered globin proteins have been found in some altitude-adapted species (14), in this case regulatory changes appear more likely. A parallel result was reported in Andean highlanders, with promoter variants at *HBB2* varying with altitude and associated with a delayed transition from fetal to adult hemoglobin (15).

Aside from *HBB*, two other anemia-associated genes were identified: *FANCA* and *PKLR*, associated with erythrocyte production and maintenance, respectively (16, 17). We also identified

genes associated with diseases linked to low oxygen during pregnancy or birth: schizophrenia (*DISC1* and *FXR6*) (18, 19) and epilepsy (*OTX1*) (20). However, the strong signal of selection affecting *DISC1*, along with *C1orf124*, might instead trace to a regulatory region of *EGLN1*, which lies between these loci (fig. S5) and functions in the hypoxia response pathway (21).

Other genes identified in this study are also located near candidate genes. *OR10X1* and *OR6Y1* are within ~60 kb of the *SPTA1* gene (fig. S5), which is associated with erythrocyte shape (22). Additionally, the three histones implicated in this study (Table 1) are clustered around *HFE* (fig. S5), a gene involved in iron storage (23). The influence of population genetic signals on neighboring genes is consistent with recent and strong selection imposed by the hypoxic environment. Stronger frequency changes at flanking genes might be expected if adaptive mutations have targeted candidate gene regulatory regions that are not near common exonic polymorphisms.

Of the genes identified here, only *EGLN1* was mentioned in a recent SNP variation study in Andean highlanders (24). This result is consistent with the physiological differences observed between Tibetan and Andean populations (25), suggesting that these populations have taken largely distinct evolutionary paths in altitude adaptation.

Several loci previously studied in Himalayan populations showed no signs of selection in our data set (table S6), whereas *EPAS1* has not been a focus of previous altitude research. Although *EPAS1* may play an important role in the oxygen regulation pathway, this gene was identified on the basis of a noncandidate population genomic survey for natural selection, illustrating the utility of evolutionary inference in revealing functionally important loci.

Given our estimate that Han and Tibetans diverged 2750 years ago and experienced subsequent migration, it appears that our focal SNP at *EPAS1* may have experienced a faster rate of frequency change than even the lactase persistence allele in northern Europe, which rose in frequency over the course of about 7500 years (26). *EPAS1* may therefore represent the strongest instance of natural selection documented in a human population, and variation at this gene appears to have had important consequences for human survival and/or reproduction in the Tibetan region.

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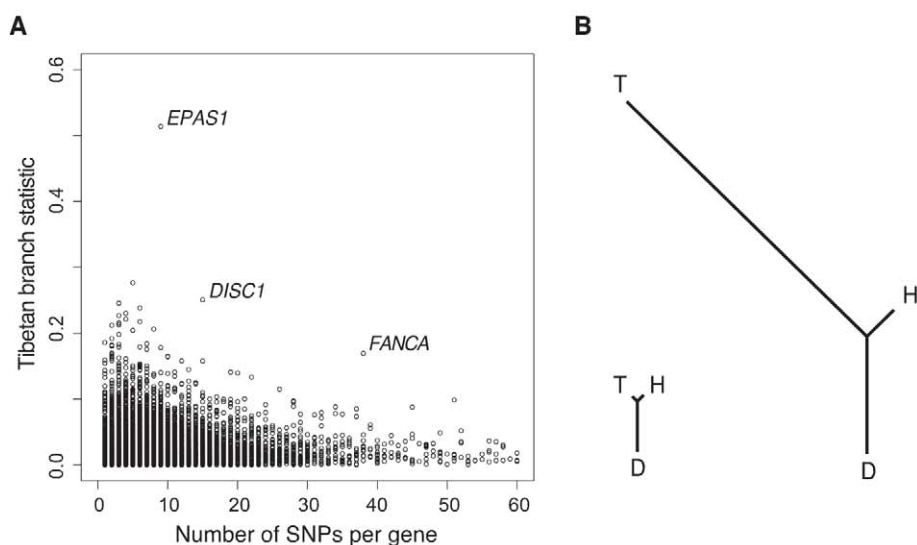


Fig. 2. Population-specific allele frequency change. (A) The distribution of F_{ST} -based PBS statistics for the Tibetan branches, according to the number of variable sites in each gene. Outlier genes are indicated in red. (B) The signal of selection on *EPAS1*: Genomic average F_{ST} -based branch lengths for Tibetan (T), Han (H), and Danish (D) branches (left) and branch lengths for *EPAS1*, indicating substantial differentiation along the Tibetan lineage (right).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/329/5987/75/DC1

Materials and Methods

Figs. S1 to S5

Tables S1 to S6

References

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Genome-Wide Reprogramming in the Mouse Germ Line Entails the Base Excision Repair Pathway

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Genome-wide active DNA demethylation in primordial germ cells (PGCs), which reprograms the epigenome for totipotency, is linked to changes in nuclear architecture, loss of histone modifications, and widespread histone replacement. Here, we show that DNA demethylation in the mouse PGCs is mechanistically linked to the appearance of single-stranded DNA (ssDNA) breaks and the activation of the base excision repair (BER) pathway, as is the case in the zygote where the paternal pronucleus undergoes active DNA demethylation shortly after fertilization. Whereas BER might be triggered by deamination of a methylcytosine (5mC), cumulative evidence indicates other mechanisms in germ cells. We demonstrate that DNA repair through BER represents a core component of genome-wide DNA demethylation in vivo and provides a mechanistic link to the extensive chromatin remodeling in developing PGCs.

The specification of mouse primordial germ cells (PGCs) at embryonic day 7.25 (E7.25) is accompanied by the initiation of epigenetic changes (1), followed by widespread epigenetic reprogramming at E11.5, which includes genome-wide DNA demethylation, erasure of genomic imprints, and large-scale chromatin remodeling (1–3) (fig. S1). Chromatin remodeling follows the onset of genome-wide DNA demethylation, which suggests that DNA repair might be linked to this process (2). DNA repair–driven DNA demethylation would involve replacement

of a methylcytosine (5mC)–containing nucleotide by an unmethylated cytosine (4, 5). As the epigenetic changes in E11.5 PGCs occur in the G₂ phase of the cell cycle and are thus independent of DNA replication (2), the most likely mechanisms for the replacement of 5mC would be the nucleotide excision repair (NER) or base excision repair (BER) pathways.

We obtained a quantitative measure of expression of BER and NER components and observed an up-regulation of transcripts of BER components *Parp1*, *Ape1*, and *Xrcc1* in E11.5 PGCs, which was not seen in the neighboring somatic cells (6). By contrast, we observed little expression of NER components *Ercc1* and *Xpa* in these PGCs (Fig. 1A and fig. S2).

Expression of ERCC1 (excision repair cross-complementing rodent repair deficiency, complementation group 1), a core NER component, occurs at low levels in PGCs and neighboring somatic cells at the time of epigenetic reprogramming compared with control ultraviolet light-irradiated primary embryonic fibroblasts (PEFs), where we observed a dose-dependent increase and nuclear localization of ERCC1 (fig.

S3). Although we detected XPA—another NER component—in both somatic cells and PGCs (fig. S4A), it was not chromatin bound and, hence, was inactive (7) (fig. S4B). Thus, the response of the NER pathway is not triggered during the reprogramming process in PGCs.

XRCC1 (x-ray repair complementing defective repair in Chinese hamster cells 1), a core component of the BER pathway (8), is present in PGC nuclei between E10.5 and E12.5 (fig. S5A), as is PARP1 [poly(ADP-ribose) polymerase family, member 1] and APE1 (apurinic/apyrimidinic endonuclease) (fig. S5, B and C). XRCC1 is a soluble nuclear factor, which binds to DNA when single-stranded DNA (ssDNA) breaks occur (8). We determined the amount of chromatin-bound XRCC1 in gonadal PGCs using the preextraction method (9). Whereas we observed an overall enrichment of XRCC1 in PGCs during E10.5 to E12.5, we found an enhancement in chromatin-bound XRCC1, specifically in PGCs at E11.5, which coincides with the stage at which genome-wide DNA demethylation occurs (Fig. 1B), which suggested that ssDNA breaks are present (10). We also detected high levels of PAR polymer, a product of activated PARP1 enzyme and an additional marker of active BER (8, 11), specifically in E11.5 PGCs (Fig. 1C). The presence of activated BER in PGCs during ongoing epigenetic reprogramming suggests that DNA demethylation in PGCs may be linked to the DNA repair pathway (2).

In PGCs spanning a period of ~6 hours, between E11.25 and E11.5, we detected increasing levels of PAR before the loss of signal for histone H1 (Fig. 1C). Because histone H1 is a target for PARP1 ribosylation (11, 12) and PARP1 itself has been shown to displace H1 (13), it is possible that high levels of PARP1-mediated poly(ADP-ribose) (PAR) synthesis in the nuclei of PGCs might be involved in H1 displacement. Additionally, we observed a correlation between high nuclear PAR signals and the disappearance of chromocenters in PGCs (fig. S5D) (2), which is consistent with a proposed role for PARP1 in the regulation of higher-order chromatin structure (11, 14, 15).

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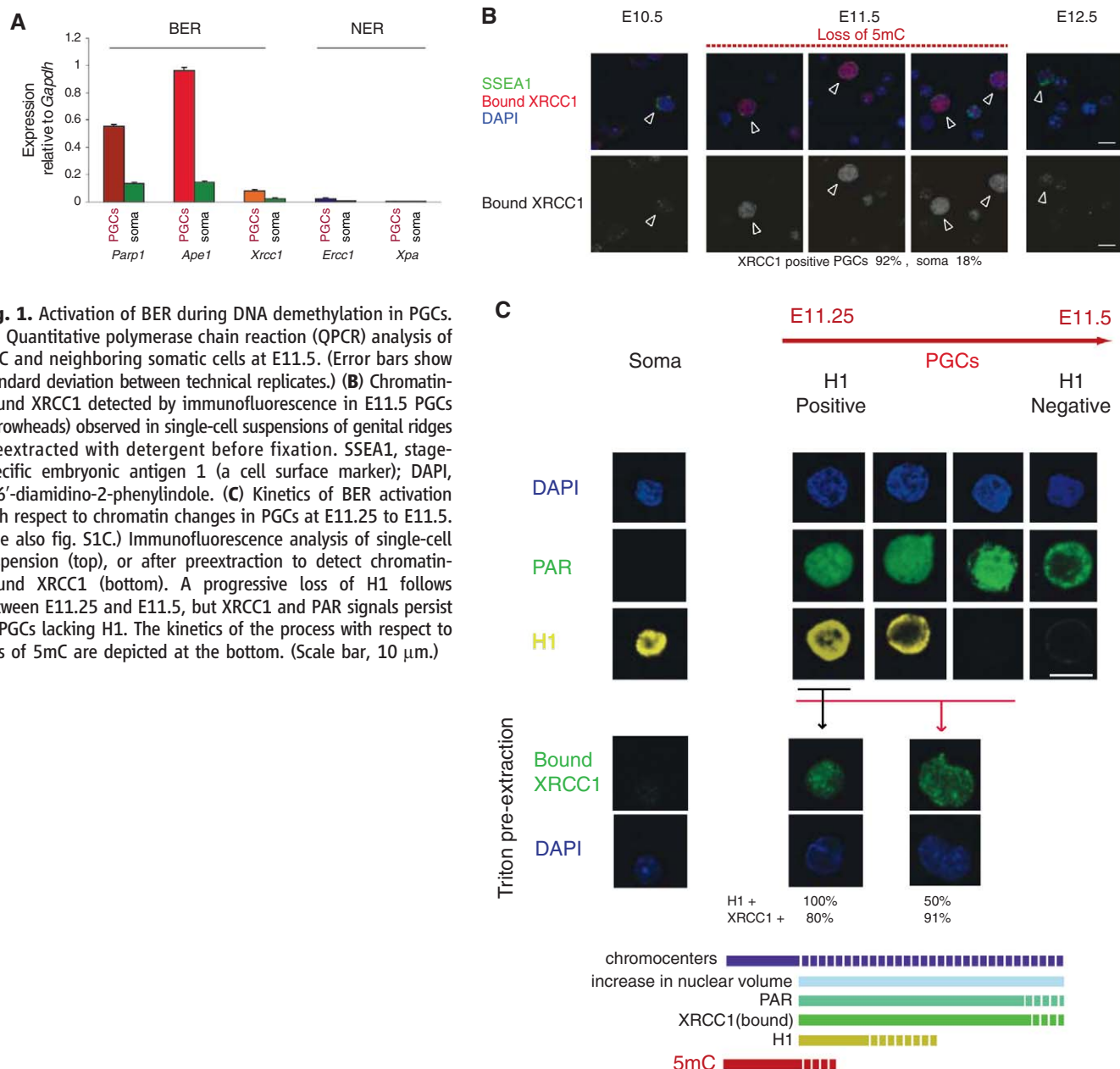
Concomitantly with the appearance of the PAR signal in the nuclei of PGCs, we detected high levels of chromatin-bound XRCC1, which suggested the presence of ssDNA breaks in nuclei of PGCs undergoing the DNA demethylation process (Fig. 1C) (10). The chromatin-bound XRCC1 was detectable in PGCs before the loss of H1, consistent with the loss of 5mC before the complete disappearance of H1 staining (2). Thus, we detect a transient population of PGCs containing PAR and chromatin-bound XRCC1 and H1, in the course of epigenetic reprogramming in PGCs (Fig. 1C).

We wished to establish whether there is a direct mechanistic link between BER and DNA demethylation. However, because PGCs are difficult to culture and manipulate in vitro, we ex-

amined mouse zygotes where there is also active genome-wide DNA demethylation that specifically affects the paternal, but not the maternal, pronucleus present in the same cell (16, 17). First, similar to our observations on PGCs, we found negligible levels of ERCC1 (fig. S6) and chromatin-bound XPA throughout zygotic development (fig. S7). In contrast, we found high levels of PARP1 and APE1 enzymes in zygotic pronuclei, as well as accumulation of PAR (figs. S8 and S9). Also, we observed high levels of XRCC1 in both parental pronuclei (Fig. 2A). However, after preextraction of soluble proteins, we detected bound XRCC1 protein only in the male pronucleus (Fig. 2B), which suggested the existence of ssDNA breaks localized to the paternal genome but not to the maternal pronucleus

in the same cell. The asymmetric chromatin-bound XRCC1 in the male genome is detectable from early pronuclear stage (PN) 3, which coincides with the onset of DNA demethylation (Fig. 2B) (17). Although we detected high levels of PAR in both the male and female pronuclei (fig. S8B), PARP1 protein was detected predominantly in the paternal pronucleus (fig. S9). It is possible that the PAR in the maternal pronucleus has a different function and is a product of a different enzyme of the PARP family.

To establish that the activation of the BER pathway was specifically associated with DNA demethylation, we wanted to exclude other possible triggers, including protamine-histone exchange and DNA replication. Protamine-histone exchange occurs in the zygote during PN1 (fig.



S10) (18), which is substantially before the onset of DNA demethylation or the detection of active BER. We also observed high levels of APE1 and chromatin-bound XRCC1 in the presence of aphidicolin, an inhibitor of replicative DNA polymerase, indicating that the process occurs independently of DNA replication (fig. S11) (16, 19, 20).

The epigenetic asymmetry of DNA demethylation in zygotes is promoted, at least in part,

by the maternal inheritance of the Stella protein (21); absence of Stella results in aberrantly targeted DNA demethylation to both the maternal and paternal pronuclei. In zygotes from *Stella* null females, the activation of BER components is detectable in both parental pronuclei. Chromatin-bound XRCC1 was clearly detectable in both pronuclei of *Stella*-depleted zygotes in contrast to the wild-type controls, where the chromatin-bound XRCC1 was predominantly confined to

the paternal pronucleus (Fig. 2C and fig. S12), which further indicated that activation of BER is linked to DNA demethylation in vivo.

We predicted that small-molecule inhibitors of key BER components should impede the progression of DNA demethylation. Addition of PARP inhibitor 3-aminobenzamide (3AB) (22), ABT-888, or the APE1 inhibitor CRT0044876 (APE1-i) (23) in the fertilization medium resulted in zygotes with significantly higher levels

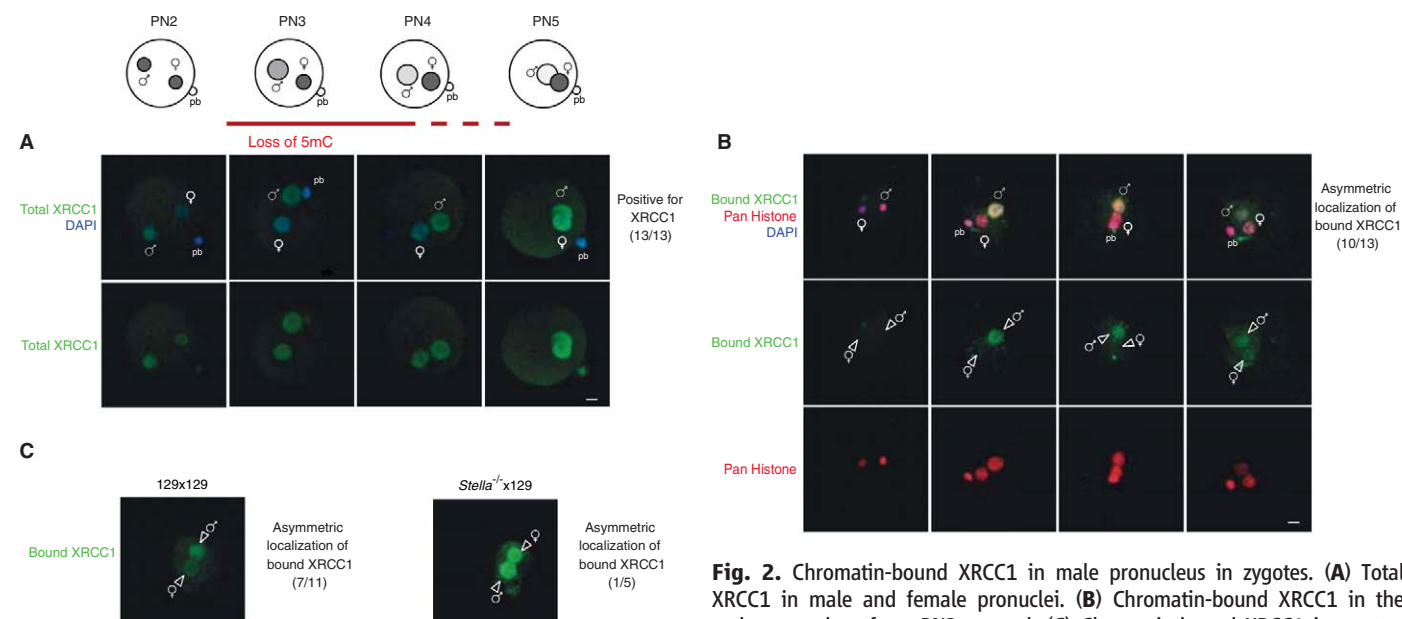


Fig. 2. Chromatin-bound XRCC1 in male pronucleus in zygotes. (A) Total XRCC1 in male and female pronuclei. (B) Chromatin-bound XRCC1 in the male pronucleus from PN3 onward. (C) Chromatin-bound XRCC1 in zygotes from wild-type and *Stella* null females. ♀, maternal pronucleus; ♂, paternal pronucleus; pb, polar body. (Scale bar, 10 μ m.)

Fig. 3. Inhibition of BER affects DNA demethylation in zygotes. (A and C) PARP inhibitor 3AB and APE1 inhibitor (CRT0044876) impede progression of DNA demethylation, as detected by 5mC staining. (B and D) Quantification of 5mC staining shown as a ratio between 5mC signal from paternal pronuclei relative to the signal from maternal pronuclei. DMSO, dimethyl sulfoxide. (Statistical analysis by Student's *t* test.) (E) Bisulfite analysis of *Line1* repetitive elements indicating the percentage of methylated CpGs. Each line represents a unique DNA clone; filled and open circles represent methylated and unmethylated CpGs, respectively. ♀, maternal pronucleus; ♂, paternal pronucleus; pb, polar body. (Scale bar, 10 μ m.)

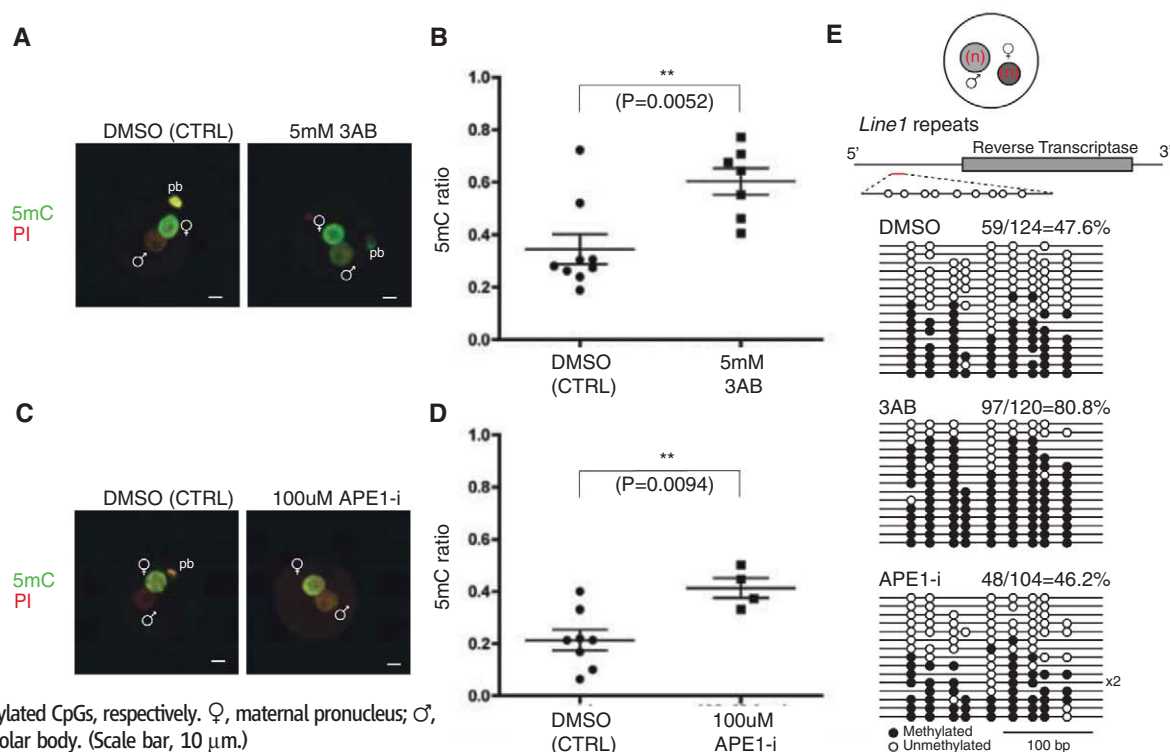
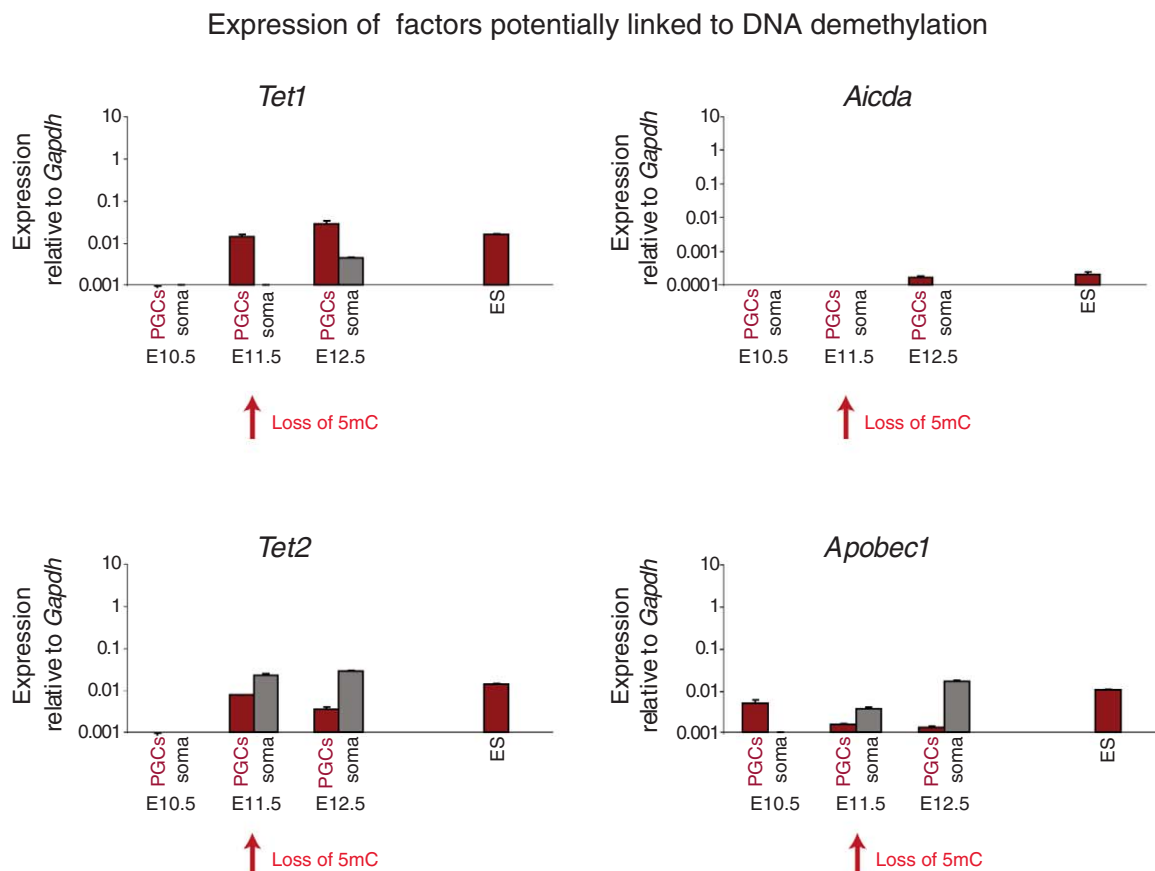


Fig. 4. Expression of 5mC-modifying enzymes and the molecular pathway of epigenetic reprogramming. Expression analysis of *Tet1* and *Tet2*, which have been implicated in 5mC to 5hmC conversion; *Tet1* shows significant expression in PGCs at E11.5 and E12.5. *Aicda* is undetectable in PGCs at the time of reprogramming, whereas the levels of *Apobec1* diminish after E10.5. (Error bars show standard deviation between technical triplicates.) The gene for glyceraldehyde-3-phosphate dehydrogenase is *Gapdh*.



of DNA methylation in the paternal pronucleus as judged by 5mC staining (Fig. 3 and fig. S13, A and B), without affecting DNA methylation levels in the maternal pronucleus. We anticipate that the presence of BER inhibitors would inhibit and lower the processivity of DNA repair, which would prevent initiation of DNA demethylation particularly on the opposite DNA strand (5) and so account for the higher levels of DNA methylation in the paternal pronucleus. This effect on DNA methylation was further confirmed by bisulfite sequencing of the repetitive *Line1* elements (Fig. 3 and fig. S13C). The use of APE1-i is likely to lead to the persistence of abasic sites in the template DNA that will prevent amplification of the affected molecules. Consistent with this, we observed amplification skewing, as judged by a shift in the relative proportions of the *Line1* subclasses in our amplicons, which may also explain an apparent lack of changes in *Line1* methylation in our bisulfite analysis. The inhibitors did not affect development of the zygotes, as judged by the rate of fertilization and progression through the pronuclear stages (PN1 to 5), and after the removal of the inhibitors, the zygotes resumed cleavage division.

We demonstrate that the genome-wide active DNA demethylation is mechanistically linked to the activation of BER and suggest that the present ssDNA breaks are likely to trigger extensive chromatin remodeling and histone exchange in PGCs (2). In plants, BER is triggered during active

DNA demethylation in response to the excision of 5mC by methylcytosine-specific DNA glycosylases (4, 24), but similar enzymes are as yet unknown in animals. Alternatively, deamination of 5mC by AID (activation-induced deaminase) or APOBEC (apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like) enzymes resulting in T-G mismatches might trigger BER and result in a loss of 5mC. However, we failed to detect expression of *Aicda* in the germ line between E8.5 and E11.5 (Fig. 4 and not shown) (25), which was consistent with the limited effect of loss of AID on DNA demethylation in PGCs (26), whereas *Apobec1* expression diminishes in PGCs before E11.5 (Fig. 4). Furthermore, either the T-G mismatch glycosylases are not consistently detectable in germ cells and zygotes (figs. S14 and S15) or the loss of function has no effect on germline development (27). We also exclude the proposed deamination driven by the DNA methyltransferases Dnmt3a or Dnmt3b (28), because E11.5 PGCs lack both these enzymes. Thus, cumulative evidence does not support a major role for 5mC deamination in germ cells. An alternative possibility includes modification of 5mC to 5-hydroxymethylcytosine (5hmC) (fig. S16) (29, 30), which could be recognized by a glycosylase. We detected significant expression of *Tet1*, which encodes a key enzyme for this modification (30) (Fig. 4), coincidentally with the transcription of BER components in E11.5 PGCs (fig. S2). However,

it is also possible that 5hmC modification plays a different role in the context of resetting the epigenome after the loss of 5mC.

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Materials and Methods

Figs. S1 to S16

References

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Increased Mutagenesis and Unique Mutation Signature Associated with Mitotic Gene Conversion

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To examine the fidelity of DNA synthesis during double-strand break (DSB) repair in *Saccharomyces cerevisiae* we studied gene conversion in which both strands of DNA are newly synthesized. The mutation rate increases up to 1400 times over spontaneous events, with a significantly different mutation signature. Especially prominent are microhomology-mediated template switches. Recombination-induced mutations are largely independent of mismatch repair, by DNA polymerases Pol ζ , Pol η , and Pol32, but result from errors made by Pol δ and Pol ϵ . These observations suggest that increased DSB frequencies in oncogene-activated mammalian cells may also increase the probability of acquiring mutations required for transition to a cancerous state.

A substantial increase in mutation rates has been proposed to explain how a single cell can sustain multiple mutations within a finite life span, resulting in malignancy (1). The origin of this mutator phenotype remains unknown, although an interesting correlation exists between increased DNA damage levels observed in oncogene-activated precancerous cells (2) and the timing of this necessary mutation rate increase. Most oncogene-induced DNA damage appears to be double-strand breaks (DSBs), which pose a severe challenge to maintaining genome integrity. Various homologous recombination (HR) mechanisms (3) repair DSBs, among which gene conversions (GCs) pose the least genomic threat. GCs have been associated with increased mutation rates (4–6), largely dependent on the error-prone translesion polymerase, Pol ζ ; however, these errors were measured in presumably single-stranded DNA adjacent to a DSB.

To directly examine GC-associated mutation rates occurring in repaired DNA consisting of two newly synthesized strands, we took advantage of budding yeast mating-type (*MAT*) switching initiated by an HO endonuclease-induced DSB and repaired by synthesis-dependent strand annealing (SDSA) (Fig. 1A) (7). We modified the heterochromatic donor *HMRa*, replacing the *HMRa1* open reading frame (ORF) with *Kluyveromyces lactis* *URA3* (*KI-URA3*) (fig. S1). DSB repair efficiency was ~99%, and nearly all repair events

yielded *Ura*⁺ (i.e., *mata1::KI-URA3*) cells. However, *Ura*[−] mutants were selected using 5-fluoroorotic acid. Cells acquiring *Ura*[−] mutations during recombination become *Ura*⁺ if *hmr::KI-URA3* remains unaltered and is desilenced (8). Among exponentially growing cells, recombination-induced *Ura*[−] mutagenesis was 240 times as high as the spontaneous rate of *mata1::KI-URA3* (Table 1) and was specific to the repaired *MAT* locus. The GC-associated mutation rate in cells synchronously released from G₁ was 5.9 times as high as asynchronous cultures ($P = 0.0001$) and 1400 times as high as spontaneous events (Table 1).

Sequences of independent *Ura*[−] GC events (Fig. 2 and Table 2) reveal that both synchronous and asynchronous wild-type (WT) cells produce mainly single base-pair substitutions (BPSs) with a significantly larger ($P = 0.006$) fraction being −1 frameshifts; these data were combined for subsequent statistical comparisons. The proportions of BPSs, −1 deletions, +1 insertions, and complex mutations differ significantly ($P = 0.015$) from our analysis of spontaneous *KI-URA3* mutations, as well as from a recent study on spontaneous mutations of *Saccharomyces cerevisiae* *URA3* (*Sc-URA3*) (9) (Table 2). Nearly all GC-associated −1 frameshifts occur in homonucleotide runs (≥ 2 bases), compared with about half among spontaneous events ($P = 0.04$) (9).

Ten out of 12 GC-associated complex mutations can be explained by template switches occurring in the context of quasipalindromes (Fig. 1, A and B, and Fig. 2); similar mutations have been observed from *Escherichia coli* to human p53 tumors (10–12). Two other complex GC events resulted from interchromosomal template

switches between the *hmr::KI-URA3* donor, on chromosome 3, and the endogenous *Sc-ura3-52* allele, on chromosome 5, which share only 73% identity. In these events the nascent DNA strand must prematurely dissociate from its donor template, *hmr::KI-URA3*, invade a new donor, *Sc-ura3-52*, and eventually come back to *hmr::KI-URA3* to complete *MAT* switching (Fig. 1A). Homology at the junctions within these *KI-Sc-KI-ura3* chimeras ranges from 2 to 11 base pairs (bp). Of 455 GC-dependent *Ura*[−] mutants sequenced in WT and several mutant backgrounds, 10 such interchromosomal template switches and 27 presumptive quasipalindrome events were identified (Table 2 and table S1). No such events were detected in spontaneous *mat::KI-URA3* mutations (Table 2). All 10 ectopic template switches used a shared 5-bp microhomology that misaligns during strand invasion and introduces a frameshift, −1 if used as the entry point or +1 if used as the exit point (Fig. 1C); all DNA synthesis after the template switch was error free. Without this misalignment, these events would apparently result in *Ura*⁺ chimeric *KI-Sc-KI-ura3* GC products and avoid detection. To generate *Ura*[−] outcomes not requiring the 5-bp misaligning sequence, we inserted two 4-bp sequences into *Sc-URA3* such that in-frame template switches would generate *Ura*[−] outcomes (fig. S4A). This modification increased interchromosomal template switch rates by a factor of about 3. Junctions contained 2 to 17 bp microhomologies, and only one of six used the 5-bp misaligning sequence (fig. S4B). We conclude that GC is considerably less processive than normal DNA replication, even though GC requires the proliferating cell nuclear antigen clamp that should anchor DNA polymerases to the template (13).

These ectopic template switches are reminiscent of spontaneous segmental duplications in yeast and copy number variation in human cancers, which are proposed to occur through microhomology-mediated break-induced replication (BIR) (14, 15) and in yeast require the non-essential Pol δ subunit, Pol32 (16). GC-associated mutation rates and mutant spectra do not change in the absence of Pol32 (Table 1, table S1, and fig. S2F). Moreover, ectopic template jumps proved to be Pol32-independent (fig. S2C); thus, we suggest that they arise by a novel microhomology-mediated SDSA pathway.

Mismatch repair (MMR) effectively repairs mismatches in heteroduplex DNA formed during *MAT*-switching strand invasion (17). However, MMR plays a minor role in discouraging mutations arising during repair DNA synthesis

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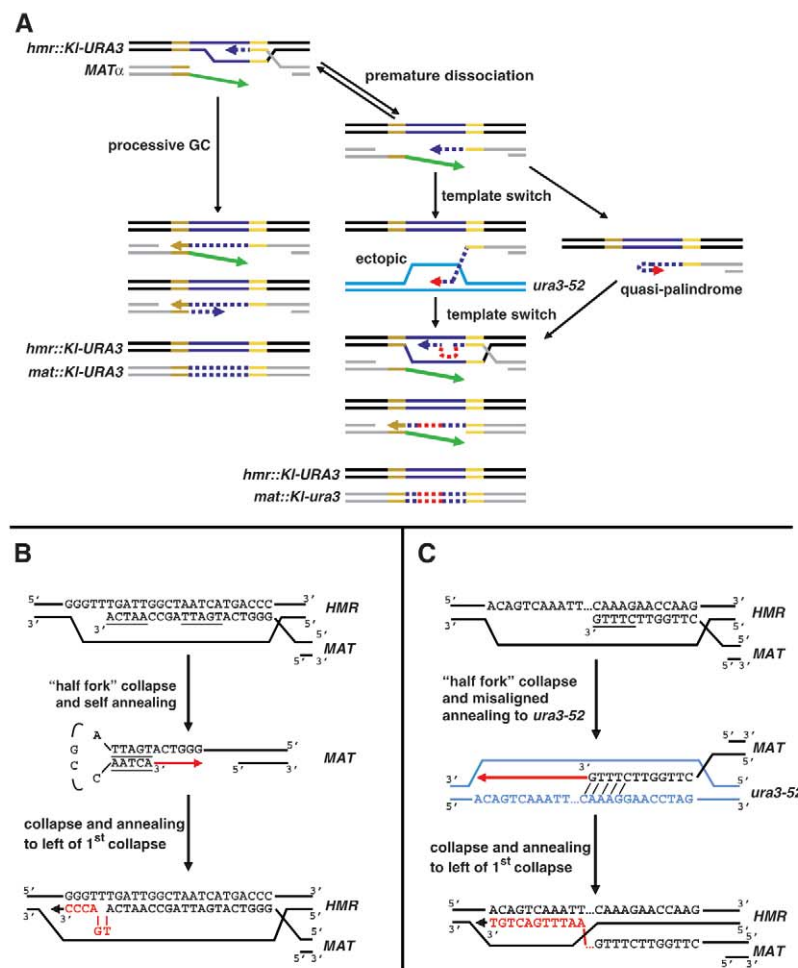


Fig. 1. (A) *MATα* switching occurs through SDSA (4). DNA synthesis beginning in *HMR-Z* copies *Y* sequences and progresses into *HMR-X*. The nascent strand dissociates and anneals to *MAT-X* sequences on the other side of the DSB. Nonhomologous sequences are clipped off, and second-strand synthesis initiates using the first strand as a template. Nonprocessive DNA synthesis results in premature dissociation from *HMR*, which can result in error-prone reannealing to the *HMR* donor template or to other ectopic templates. To complete the GC, template switch events must dissociate again and reanneal to *HMR*. **(B)** GC-associated quasipalindrome template switch at position 584-585 within the *KI-URA3* ORF. **(C)** GC-associated ectopic template switch into *Sc-ura3-52* and then back to *hmr::KI-URA3* in which a frameshift is introduced due to misalignment at a shared 5-bp sequence. Underlined sequences indicate *KI-URA3* microhomology (nucleotides 423 to 427).

Table 1. Spontaneous and GC-dependent mutation rates. Relative changes from the corresponding WT rate are in parentheses. *, $P < 0.05$ (t-test) versus the corresponding WT rate; **, $P < 0.01$; ***, $P < 0.001$; ±, SEM; n/a, not applicable.

Genotype	Spontaneous mutation rate ($\times 10^{-8}$)	<i>MAT</i> -switching mutation rate ($\times 10^{-5}$)	Relative change in rate from spontaneous
WT	5.8 ± 2.3 (1)	1.4 ± 0.5 (1)	240
WT G1/S	n/a	8.1 ± 1.0 (5.9)***	1400
<i>mlh1Δ</i>	239 ± 46 (41)***	5.6 ± 1.4 (4)**	23
<i>msh6Δ</i>	229 ± 42 (39)***	2.3 ± 0.35 (1.6)	10
<i>rev3Δ</i>	8.1 ± 2.4 (1.4)	1.6 ± 0.31(1.1)	200
<i>rev3Δrad30Δ</i>	10.1 ± 2.3 (1.7)	3.2 ± 0.66 (2.3)**	310
<i>pol32Δ</i>	20 ± 7.5 (3)*	0.66 ± 0.5 (0.5)	33
<i>pol30(K127,164R)</i>	4.6 ± 0.02 (1)	2.9 ± 0.3 (2.1)	630
<i>pol2-4</i>	43 ± 11 (7)**	6.0 ± 0.78 (4.3)**	140
<i>pol3-01</i>	440 ± 35 (76)**	3.1 ± 1.0 (2.2)	7
<i>dun1Δ</i>	15 ± 6 (3)	2.0 ± 0.6 (1.5)	130

(Table 1). Moreover, mutant spectra of *mlh1Δ* and *msh6Δ* strains were not different from WT (figs. S2B and S2C and table S1). Thus, either the extending DNA strand does not recruit MMR proteins during GC or MMR cannot distinguish old from new strands, and, hence, mutations are as likely to be introduced as removed.

Error-prone Rev3 (DNA Polζ) appears to play a central role in generating BPSs but not frame-shift mutations in presumably single-stranded DNA adjacent to a repaired DSB (4–6). We investigated the role for *REV3* where both DNA strands are newly synthesized. *REV3* does not significantly affect recombination efficiency or the GC mutation rate or spectrum (Table 1, table S1 and fig. S1D). We suggest that Rev3 plays a nonessential role in filling-in single-strand gaps adjacent to the repaired locus but not within the gap-repair region. We also created a *rev3Δ rad30Δ* double mutant, lacking both Polζ and the error-prone polymerase Polη (18). *MAT*-switching efficiency and mutant spectra were not affected, but there was a modest, statistically significant increase in the GC mutation rate (Table 1 and fig. S1E), suggesting that Polη plays an error-free role during GC. Further evidence that translesion synthesis (TLS) polymerases do not greatly affect GCs is illustrated by the observation that GC mutation rates are not changed by *pol30-K127,164R*, an allele that prevents posttranslational modification by ubiquitylation or the addition of the small ubiquitin-like modifier, SUMO, modifications that recruit TLS polymerases (Table 1).

Both Polδ and Pole DNA polymerases are important for HR (13, 16, 19–23). To test for preferential usage during GC, we examined proofreading deficient, error-prone alleles, *pol3-01* and *pol2-4*, respectively. The GC mutation rate for *pol2-4* is 4.3 times as high as WT ($P = 0.004$), indicating that Pole plays an important replicative role during GC (Table 1). The spontaneous and GC mutation spectra for *pol2-4* were significantly different from WT with a large increase in +1 frameshifts (Table 2 and Fig. S1, G and H).

The GC-dependent mutation rate for *pol3-01* was only 2.2 times as high as WT ($P > 0.05$), suggesting that Polδ plays a less substantial role during GC than previously suggested (22); however, the Ura⁺ mutation spectrum revealed that Polδ plays a major role. Most striking, *pol3-01* eliminated all –1 frameshifts and complex events ($P < 0.0001$) (Table 2 and fig. S1I), which also occurred among spontaneous mutations ($P < 0.05$) (Table 2 and fig. S1J). The lack of –1 frameshifts and other template switches may be explained by the fact that a Polδ proofreading-defective protein is actually more processive than WT (24). Much of the increased spontaneous mutation rate in *pol3-01* appears to be a consequence of activating a Dun1 kinase-dependent S-phase or DNA damage checkpoint (25). It is possible that the GC-associated effects with *pol3-01* could be the result of activating a Dun1-

Table 2. Spontaneous and GC-dependent spectra for *K. lactis URA3*. For 1-bp deletions, 1-bp insertions, BPSSs, and complex mutations, numbers in parentheses indicate percentage of total mutations represented by each respective

mutation. –1 deletions and +1 insertions are divided into events occurring in either homonucleotide runs ≥2 nucleotides (nt) or nonrepeating sequences. spont., spontaneous; comb., combined data from asynchronous and G1 cells.

Type of mutation	WT spont.	<i>Sc-URA3</i> spont. (9)	WT GC comb.	<i>pol2-4</i> GC	<i>pol2-4</i> spont.	<i>pol3-01</i> GC	<i>pol3-01</i> spont.
bp substitutions	46 (75%)	167 (81%)	56 (54%)	21 (46%)	41 (82%)	48 (98%)	49 (98%)
Transitions	19	46	32	10	8	20	17
Tranversions	27	121	24	11	33	28	32
–1 deletions	5 (8.0%)	22 (11%)	32 (31%)	12 (26%)	0	0	1 (2.0%)
–1 in homo nt	3	14	29	12	0	0	0
–1 in mono nt	2	8	3	0	0	0	1
+1 insertions	1 (2.0%)	3 (1.0%)	1 (1.0%)	7 (15%)	9 (18%)	1 (2.0%)	0
+1 in homo nt	1	2	1	7	9	1	0
+1 in mono nt	0	1	0	0	0	0	0
Template switches	0	n/a	12 (12%)	5 (11%)	0	0	0
Ectopic (<i>Sc-ura3-52</i>)	0	n/a	2	2	0	0	0
Quasipalindrome	0	n/a	10	3	0	0	0
Other complex	9 (15%)	15 (7.0%)	2 (2.0%)	1 (2.0%)	0	0	0
Total mutations	61	207	104	46	50	49	50

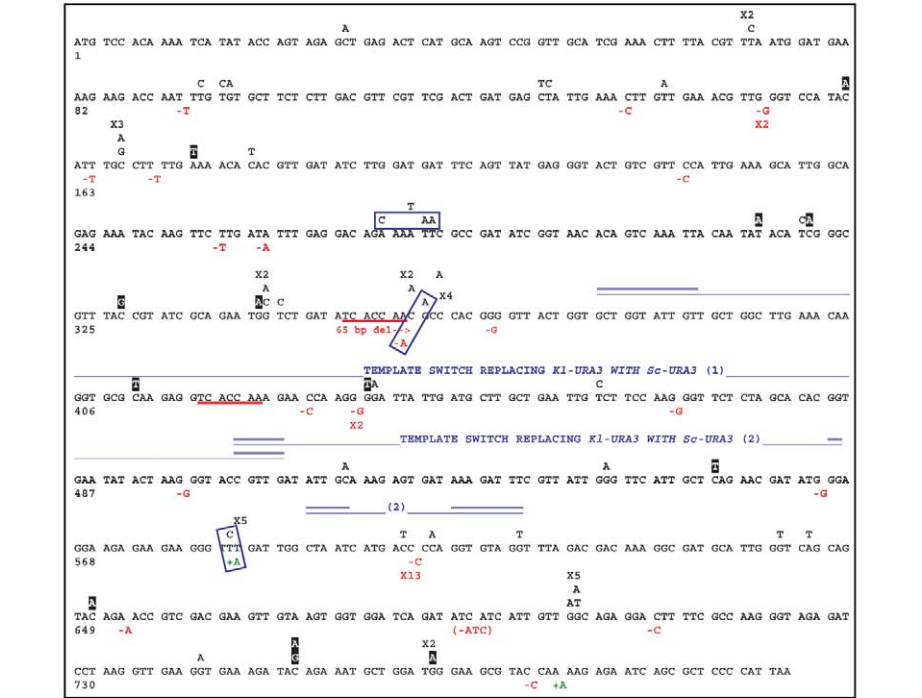


Fig. 2. GC-associated *KI-URA3* mutant spectrum. Single letters above the *KI-URA3* ORF indicate BPSSs. Black boxes with white text indicate nonsense mutations. Insertions and deletions are shown below the ORF; insertions are green and deletions are red. A large deletion occurring between direct repeats is indicated with repeats underlined in red. Blue lines below the ORF mark two ectopic events where *KI-URA3* sequences were replaced by *Sc-ura3-52* sequences; double blue lines depict microhomology junctions. The template switch marked as “(2)” contained two independent jumps to *Sc-ura3-52*, one of which contained a frameshift. Quasipalindrome-mediated template switch mutations are within blue boxes (Fig. 1C and fig. S3 depict these mechanisms). Recurrent mutations are indicated by an X followed by the number of times that mutation was observed.

dependent checkpoint; however, we find that deleting Dun1 alone has no effect on the rate of spontaneous or GC mutation rates or mutant spectra (Table 1, table S1, and fig. S2K).

We conclude that Pole and Polδ are both involved in DNA synthesis during GC, with no apparent preference for using one polymerase over

the other. Whether first- and second-strand syntheses are carried out by different DNA polymerases, we cannot ascertain. The presence of frequent template switches suggests that DNA synthesis during GC has decreased processivity compared with S-phase replication. During a nonprocessive GC where there is premature dis-

sociation of the nascent DNA strand from the template, followed by reinitiation, there may be an opportunity to switch between polymerases. Although GC is generally thought to be the most error-free pathway of DSB repair, it exhibits a significantly elevated mutation rate. Weinberg (26) argues that mammalian cells must suffer ≥6 mutations before transitioning to a fully cancerous state. How cells experience such high mutation levels during limited life spans remains unknown, except when MMR genes or proof-reading activity of replicative polymerases are mutated (27–29). The observation that activated oncogenes promote dramatic increases in DSB formation (2) suggests that some mutations required for carcinogenesis may arise by increased recombination-associated mutation rates.

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Supporting Online Material

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Materials and Methods

Figs. S1 to S4

Table S1

References

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Reprogramming of T Cells to Natural Killer–Like Cells upon *Bcl11b* Deletion

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T cells develop in the thymus and are critical for adaptive immunity. Natural killer (NK) lymphocytes constitute an essential component of the innate immune system in tumor surveillance, reproduction, and defense against microbes and viruses. Here, we show that the transcription factor *Bcl11b* was expressed in all T cell compartments and was indispensable for T lineage development. When *Bcl11b* was deleted, T cells from all developmental stages acquired NK cell properties and concomitantly lost or decreased T cell–associated gene expression. These induced T-to–natural killer (ITNK) cells, which were morphologically and genetically similar to conventional NK cells, killed tumor cells in vitro, and effectively prevented tumor metastasis in vivo. Therefore, ITNKs may represent a new cell source for cell-based therapies.

T cell development involves progenitor homing, lineage specification, and commitment and requires a complex interplay among key transcription factors (1, 2). In the periphery, the cytokine interleukin 7 (IL-7) and the constant interaction of T cells with self peptide–major histocompatibility complex (MHC) play a critical role in T cell maintenance (3). Reverse transcription–polymerase chain reaction (RT-PCR) analysis indicates that many genes important for T cell commitment start to increase their expression in

the transition through the double-negative (DN) stages from DN1 to DN2, and *Bcl11b* is the most up-regulated transcription factor (4). In bony fish, *Bcl11b* is shown to be required for T cell–precursor homing to the thymus (5). In the mouse, *Bcl11b* has critical roles in fetal thymocyte development and survival, for positive selection, and in survival of double-positive (DP) thymocytes (6, 7).

To determine *Bcl11b* expression in T cells at the single-cell level, we produced and analyzed a *Bcl11b*-tdTomato knock-in mouse (fig. S1, A and B) (8). In hematopoietic lineages, *Bcl11b* was not expressed in B or myeloid cells, whereas almost all DN2 to DN4 and DP thymocytes, CD4⁺ and CD8⁺ T cells, $\gamma\delta$ T cells, and natural killer T cells (NKTs) expressed *Bcl11b* (figs. S2, A to C, and S3, A to C). In DN1 thymocytes, very little to no expression of *Bcl11b* was detected in CD117⁺ cells [known as early T cell lineage progenitors (2)] (figs. S2A and S3A). During NK development, transient low *Bcl11b* expression was observed in immature NK cells but not in NK precursors or mature NK cells (figs. S2D and S3D). In contrast, the majority of thymic NK cells, identified by CD127 (9), expressed *Bcl11b* (figs. S2D and S3E). Moreover, using quantitative real-time PCR (QRT-PCR) analysis, we showed that, in both CD4⁺ and CD8⁺ splenic T cells, *Bcl11b* transcription in naïve (CD44⁺CD62L⁺)

T cells was roughly two times that in activated (CD44⁺CD62L⁺) T cells (figs. S2E and S3F), and activated T cells exhibited a bimodal pattern of expression (fig. S2F).

To further determine *Bcl11b* functions in T cells, we generated the conditional knockout mice (*Bcl11b*^{flox/flox}) (fig. S4A), which were crossed to the *Rosa26Cre-ERT2* mice (10). Consequently, in *CreERT2*; *Bcl11b*^{flox/flox} mice (the PLBD line, referred to hereafter as *flox/flox*), *Bcl11b* could be deleted by treating cultured cells or mice with 4-hydroxytamoxifen (OHT). Using OHT-treated whole thymocytes from these and the control (*CreERT2*; *Bcl11b*^{flox/+}, referred to as *flox/+*) mice, we sorted and subsequently cultured DN1 and DN2 cells in T cell media on OP9-DL1 stromal cells (fig. S4B) (11), which support T cell development but suppress NK cell development from the progenitors (12). All stromal cells were killed in the OHT-treated *flox/flox* DN1 thymocyte culture.

Flow cytometry showed that 24% of cells in this culture expressed NKp46, which is primarily expressed on NK cells (Fig. 1A) (13). These NKp46⁺ cells did not express T cell genes for CD3 or the β T cell receptor (TCR β) (fig. S4C) and had lost both alleles of the *Bcl11b* exon 4 (fig. S4D), which indicated that they did not acquire or had lost T cell features, even though they were cocultured with OP9-DL1 stromal cells for 14 days. However, the control OHT-treated *flox/+* and untreated *flox/flox* DN1 cells proliferated rapidly, and many acquired CD3 expression but not NKp46⁺ (Fig. 1A and fig. S4E). These data demonstrated that *Bcl11b* deficiency caused production of the NKp46⁺ cells from DN1 thymocytes and that *Bcl11b* was required early in T cell development. Similar to cultured DN1 thymocytes, OHT-treated *flox/flox* DN2 thymocytes also produced NKp46⁺CD3⁺ cells, which killed the stromal cells, whereas control DN2 thymocytes did not (Fig. 1A and fig. S4E). Growth of NK-like cells from *Bcl11b*-deficient DN1 or DN2 thymocytes appeared to be independent of Notch signaling, because NKp46⁺ cells were readily produced from DN1 or DN2 thymocytes cultured on OP9 stromal cells without IL-2 or IL-15 (fig. S4F).

We subsequently deleted *Bcl11b* in DN3 thymocytes. Again, stromal cell-killing NKp46⁺CD3⁺ cells appeared (Fig. 1, B and C, and fig. S4G). The reprogramming also worked in myeloid or B cell culture media (fig. S4, H and I), which demonstrated that reprogramming to NKp46⁺ cells

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was intrinsic to the *Bcl11b*-deficient thymocytes. To confirm the T cell origin of NKp46⁺CD3⁺ cells, we examined their *TCRβ* locus. These cells exhibited TCRβ V(D)J recombination [recombination of the variable (V), diversity (D), and joining (J) gene segments] even though TCRβ was not expressed (Fig. 1D). We therefore named these killer cells that were reprogrammed from T cells induced T-to-natural killer or ITNK cells.

We next compared, using microarray analysis, the expression profiles of DN3 thymocytes; normal splenic NK cells that were expanded in vitro after enrichment (lymphokine-activated killer, or LAK cells, composed of >90% NK cells); and ITNKs reprogrammed from DN3 cells (Fig. 1E). Consistent with the killing ability of ITNK cells, their expression profile was more similar to that of LAK cells than to their parental DN3 thymocytes. QRT-PCR validation showed that expression of many T-lineage genes—such as *Notch1*, *Ets1*, *Hes1*, *Gata3*, *Dtx1*, and *Tcf1*—was decreased, whereas expression of genes usually associated with NK cells—such as *Id2* (14),

IL2rβ (CD122), *Zfp105* (15), and *E4bp4* (16)—was up-regulated (Fig. 1F and table S1). *Zbtb32* (Rog, Repressor of GATA), which is not normally expressed in DN3 cells but plays important roles in regulating T cell activation and suppresses Gata3 activity (17), was highly expressed in ITNKs. Expression of *Cdkn1c* (*p57KIP2*), a putative direct downstream target gene of *Bcl11b* (18), was also drastically increased in ITNKs (Fig. 1F). These results collectively demonstrated that *Bcl11b* was essential for maintaining the T cell expression profile and for suppressing NK cell gene expression.

ITNKs could also be produced from mature T cells. We OHT-treated sorted DP thymocytes, CD4⁺ and CD8⁺ T cells, and γδ T cells from *flox/flox* mice. Many ITNKs (NKp46⁺) were found growing in DP thymocytes and CD8⁺ T cell cultures (fig. S4, K and L), which effectively killed stromal cells. These ITNKs, in contrast to those reprogrammed from DN1 to DN3 thymocytes, retained TCRβ on the cell surface. We were unable to obtain consistent production of NKp46⁺ cells from splenic or thymic CD4⁺ T cells or from γδ

T cells, because these cells appeared prone to cell death in vitro once *Bcl11b* was deleted.

To estimate the reprogramming efficiency, we sorted single DN3 thymocytes from OHT-treated *flox/flox* thymocytes into individual wells of 96-well plates preseeded with OP9-DL1 stromal cells in T cell medium (fig. S5A). Out of the 79 wells that had cells growing, 36 wells had many fast-proliferating T cells (Fig. 2A), which deleted only one *flox Bcl11b* allele (Fig. 2B, lanes T1 and T2). These cells (*flox*^{−/−}) nevertheless served as excellent controls for Cre toxicity because they had activated Cre recombinase. In the other 43 wells, thymocytes were reprogrammed to NKp46⁺ stromal cell-killing ITNKs (Fig. 2A). IL-2 was clearly able to greatly promote proliferation of ITNKs, because, from one DN3 thymocyte, up to 0.5 million ITNKs were obtained with IL-2, but only ~50,000 cells without IL-2. All ITNK cells had lost both *Bcl11b* alleles (Fig. 2B, lanes I1 and I2), and ITNKs of individual wells had unique rearranged TCRβ loci, which confirmed their independent origins (Fig. 2C). Therefore, once

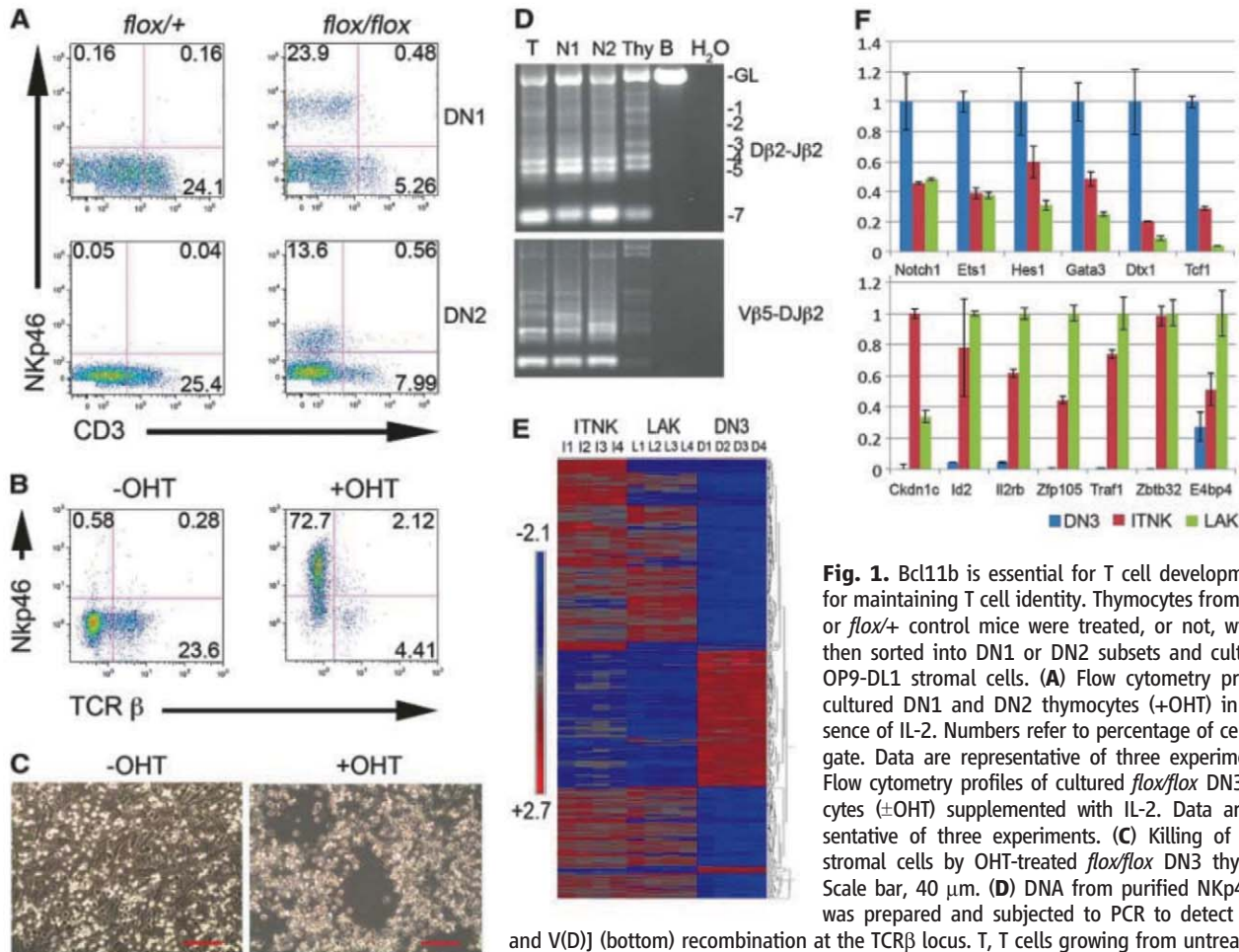


Fig. 1. *Bcl11b* is essential for T cell development and for maintaining T cell identity. Thymocytes from *flox/flox* or *flox*^{+/+} control mice were treated, or not, with OHT, then sorted into DN1 or DN2 subsets and cultured on OP9-DL1 stromal cells. (A) Flow cytometry profiles of cultured DN1 and DN2 thymocytes (±OHT) in the absence of IL-2. Numbers refer to percentage of cells in the gate. Data are representative of three experiments. (B) Flow cytometry profiles of cultured *flox/flox* DN3 thymocytes (±OHT) supplemented with IL-2. Data are representative of three experiments. (C) Killing of OP9-DL1 stromal cells by OHT-treated *flox/flox* DN3 thymocytes. Scale bar, 40 μm. (D) DNA from purified NKp46⁺ cells was prepared and subjected to PCR to detect DJ (top) and V(D)J (bottom) recombination at the TCRβ locus. T, T cells growing from untreated DN3 thymocytes; N1 and N2, sorted NKp46⁺ cells growing from OHT-treated *flox/flox* DN3 thymocytes; Thy, wild-type whole thymocytes; B, B cells; and GL, germline band. H₂O: no DNA template in PCR. Numbers indicate DJ recombination products. (E and F) Microarray analysis of gene expression in NKp46⁺CD3⁺ ITNK cells from DN3 thymocytes (columns I1 to I4), IL-2-expanded NK cells (LAK; L1 to L4) and sorted DN3 *flox/flox* thymocytes (DN3; D1 to D4) were subjected to expression. (E) Two-way hierarchical cluster map of the array data. Column numbers (I1 to I4 for instance) refer to four independent RNA samples for each cell type, and rows represent individual transcripts. Scale indicates the log₂ value of normalized signal level. (F) QRT-PCR validation of gene expression of selected genes among ITNKs, LAKs, and DN3 cells. Bars are means ± SD of three samples.

Bcl11b was deleted, the reprogramming efficiency of DN3 thymocytes to ITNKs could reach 100%. ITNKs from DN3 thymocytes not only expressed NK cell surface receptors and had similar cytotoxic functions, but were morphologically similar to LAK cells. ITNKs were larger than thymocytes and had granules and showed evidence of high protein-synthesis activity with abundant endoplasmic reticulum (Fig. 2, D and E). Besides Nkp46, ITNKs expressed NKG2A/C/E, TRAIL, perforin, and interferon- γ , but not some other key NK cell function genes, such as members of the Ly49 family (fig. S5, B and C). Similar observations were made with in vitro reprogrammed ITNK cells from DP thymocytes (table S2 and fig. S5D). ITNKs were unlikely to be related to thymic NK cells because they did not express CD127 (fig. S5E). Moreover, unlike conventional mature NK cells, most ITNKs did not express CD11b, rather, they expressed CD27 and retained their killing ability even after being cultured in vitro for 1 month (fig. S5F).

We next measured the killing ability of the DN3-reprogrammed ITNKs by performing standard ^{51}Cr -release assays with three cell lines: B16F10 melanoma, which are MHC class I (MHC I) low or negative (19); RMA lymphoma, which express MHC I; and RMA-S lymphoma (TAP-1-deficient variant), which have reduced MHC I presentation (20, 21). LAK cells generally only killed MHC I-negative cells (Fig. 2F). Similar to LAKs, ITNKs

also selectively killed MHC I-negative B16F10 and RMA-S cells, although they had slightly lower killing potency (Fig. 2F).

To exclude the possibility that ITNKs were in vitro artifacts, we deleted *Bcl11b* in vivo (fig. S6A). Two to 3 weeks after OHT treatment, ITNKs were detected in both the spleen (Nkp46 $^{+}$ CD3 $^{+}$) and the thymus (Nkp46 $^{+}$) from *flox/flox* mice but not from the *flox/+* controls (Fig. 3A). *Bcl11b* was found deleted in these in vivo reprogrammed ITNKs (fig. S6B). Both CD4 $^{+}$ and CD8 $^{+}$ ITNKs (Nkp46 $^{+}$) were found (fig. S6C). Some wild-type $\gamma\delta$ T cells expressed Nkp46; however, *Bcl11b* deletion caused a threefold increase in the Nkp46 $^{+}$ $\gamma\delta$ T cells (Fig. 3B), which suggested that all T cell populations have reprogramming potential. The in vivo reprogrammed ITNKs could readily be expanded in NK culture conditions (fig. S6D), but they were not NKT cells (fig. S6, E and F). Besides expressing NK cell-associated genes, the in vivo reprogrammed ITNKs also lost or decreased some key T cell genes such as *Il7r*, *Tbx21* (*T-bet*), and *Cd8a* (fig. S6G). Consequently, TCR signaling in ITNKs appeared to be compromised (fig. S6H).

The in vivo analysis of ITNKs in *flox/flox* mice was complicated by the presence of many host T cells and NK cells (Fig. 3A). To address this problem and also to investigate whether in vivo reprogramming upon *Bcl11b* loss is cell autonomous, we transplanted OHT-treated DP

thymocytes from *flox/flox* mice (CD45.2 $^{+}$) into *Rag2* $^{-/-}$ *Il2rg* $^{-/-}$ mice (CD45.1 $^{+}$) that lack B, T, and NK cells (fig. S7A) (22). We chose DP thymocytes because they usually account for more than 75% of total thymocytes and could be efficiently reprogrammed in vitro to ITNKs (fig. S4K). Two weeks after transplantation, ~5% of splenocytes were found to be from the donor cells (CD45.2 $^{+}$) (Fig. 3C), and ~47% of them expressed Nkp46 and thus were ITNKs. ITNKs lost both copies of *Bcl11b*, and the majority of them expressed CD8 (fig. S7, B and C). The other 53% of cells (Nkp46 $^{-}$) were T cells and still retained the *Bcl11b* floxed allele (fig. S7C). ITNKs were also found in the bone marrow and peripheral blood (fig. S7D). We estimated that there were ~200,000 ITNK cells in the spleen. No Nkp46 $^{+}$ cells were found in control mice transplanted with untreated DP thymocytes (Fig. 3C). ITNK cells were maintained in the recipients for at least 3 months without change in cell number, perhaps representing a dynamic balance in their numbers. The recipient mice did not show any noticeable abnormality, which indicated that ITNK cells did not indiscriminately kill normal cells, nor were they malignantly transformed.

Compared with those reprogrammed in vitro, the in vivo reprogrammed ITNKs expressed NK surface receptors of the Ly49 family, including Ly49C/I and Ly49G2 (fig. S7E) (table S2), and

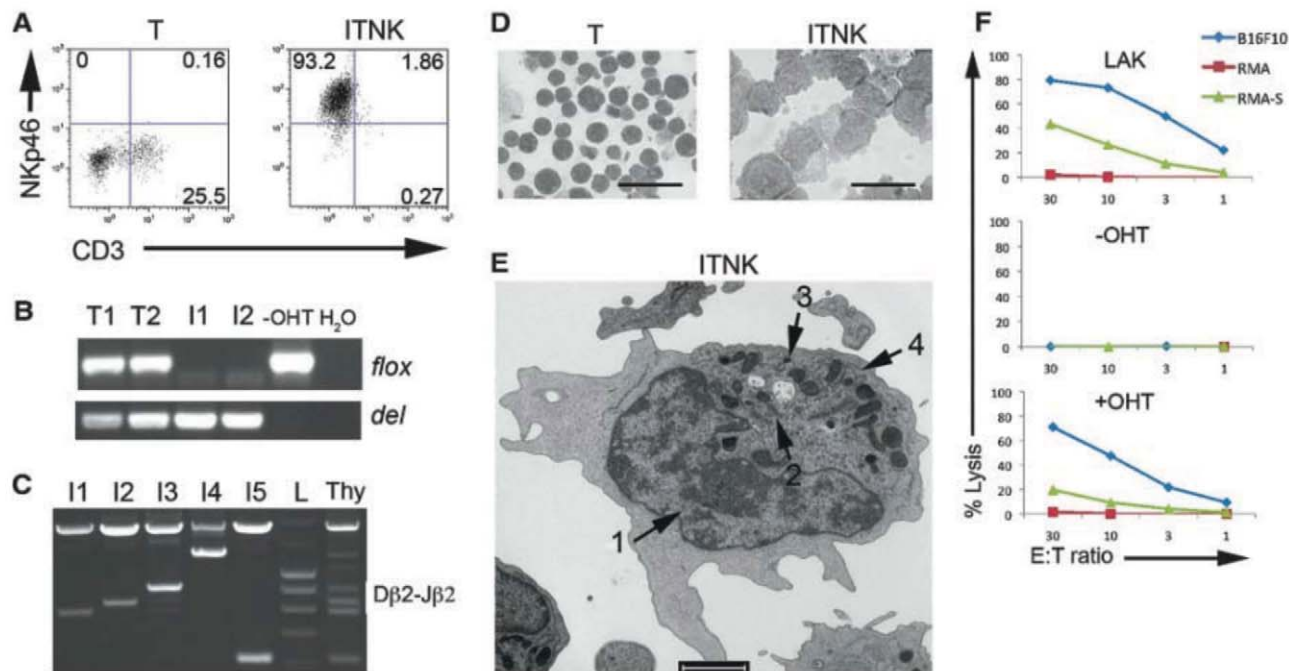


Fig. 2. Efficient reprogramming of T cells to ITNKs. (A) Representative flow cytometry profiles of ITNKs reprogrammed from single *flox/flox* DN3 cells. Numbers refer to percentage in total cells. T: T cells that did not have complete *Bcl11b* deletion. Data are representative of three experiments. (B) PCR genotyping of *Bcl11b* deletion in two representative T cell (T1 and T2) and ITNK (I1 and I2) wells. *flox*, floxed allele; *del*, deletion allele. -OHT: no OHT treatment; H₂O: no template control. (C) DJ recombination at the TCR β locus of five ITNK wells (I1 to I5) showing unique DJ

recombination. L, DNA ladder; Thy, wild-type thymocytes. (D) Giemsa stain of parental DN3 thymocytes (T) and ITNK cells. Scale bar, 20 μm . (E) Transmission electron micrograph of an ITNK cell. 1, Nucleus; 2, Golgi body; 3, granule; 4, endoplasmic reticulum. Scale bar, 2 μm . (F) Cytotoxicity of ITNKs (labeled as "+OHT") and LAKs measured in standard ^{51}Cr -release assays with B16F10, RMA, and RMA-S tumor cell targets at the indicated effector-to-target (E:T) ratios. -OHT: *flox/flox* T cells. Data are means of triplicate wells.

could be extensively expanded ex vivo with IL-2 or IL-15 for at least 3 weeks while still retaining their killing ability (Fig. 3D).

The ex vivo expanded ITNKs were then assessed for their tumor cell-killing capability. These cells exhibited elevated cytotoxic potential and

were also generally more potent than both in vitro ITNKs and LAKs against each of the target cells (Fig. 3E and Fig. 2F). Moreover, they killed RMA

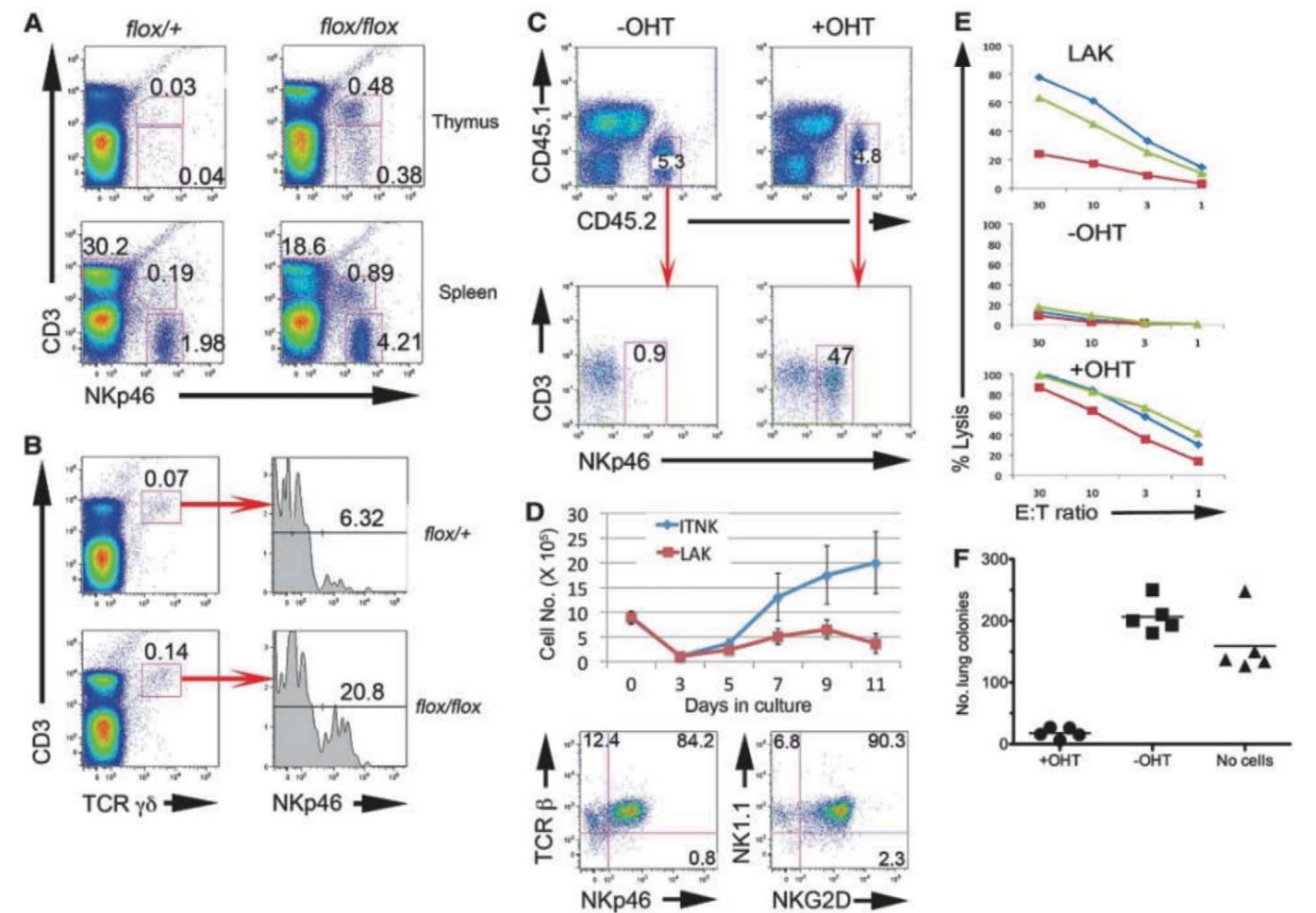


Fig. 3. ITNKs reprogrammed in vivo are potent tumor cell killers. (A) Flow cytometric analysis of thymocytes and splenocytes from OHT-treated *flox/flox* and *flox/+* mice. Numbers refer to the percentage in the lymphocyte gate. Data are representative of four mice. (B) Analysis of ITNKs from thymic $\gamma\delta$ T cells in OHT-treated *flox/flox* mice. Data are representative of two mice. (C) ITNKs production in *Rag2^{-/-}Il2rg^{-/-}* recipients injected with *flox/flox* DP thymocytes. Two weeks after injection, donor (CD45.2⁺) and host (CD45.1⁺) splenocytes were analyzed. Numbers refer to the percentage of lymphocyte gate. Plots are representative of 15 mice from three independent experiments. (D) Ex vivo expansion of ITNKs in IL-2 from splenocytes of the recipient mice. Viable cells were counted (top) at the indicated time points and analyzed (bottom). Numbers refer to percentages. Most cells in the culture

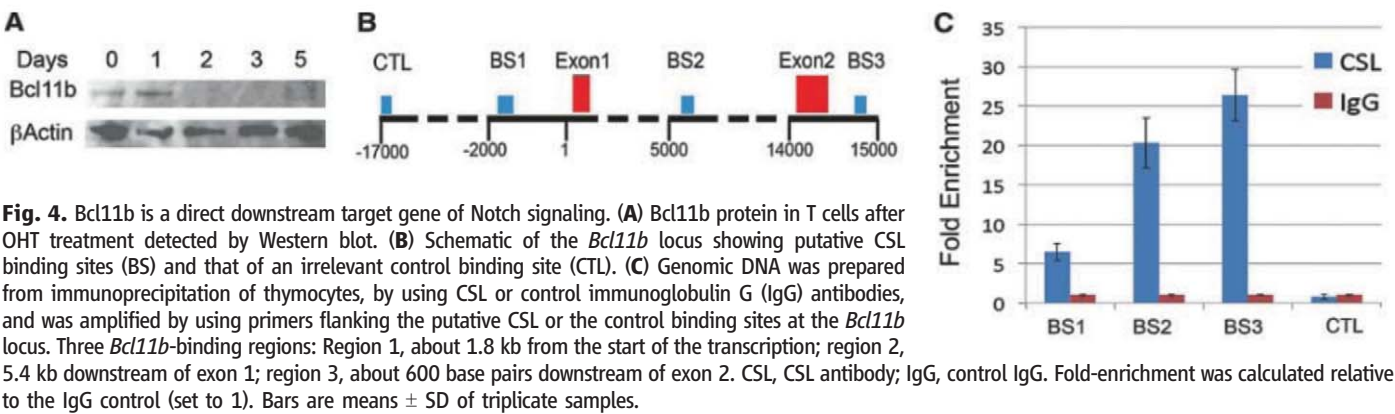


Fig. 4. Bcl11b is a direct downstream target gene of Notch signaling. (A) Bcl11b protein in T cells after OHT treatment detected by Western blot. (B) Schematic of the *Bcl11b* locus showing putative CSL binding sites (BS) and that of an irrelevant control binding site (CTL). (C) Genomic DNA was prepared from immunoprecipitation of thymocytes, by using CSL or control immunoglobulin G (IgG) antibodies, and was amplified by using primers flanking the putative CSL or the control binding sites at the *Bcl11b* locus. Three *Bcl11b*-binding regions: Region 1, about 1.8 kb from the start of the transcription; region 2, 5.4 kb downstream of exon 1; region 3, about 600 base pairs downstream of exon 2. CSL, CSL antibody; IgG, control IgG. Fold-enrichment was calculated relative to the IgG control (set to 1). Bars are means $\pm$ SD of triplicate samples.

cells with almost the same efficiency as they killed RMA-S cells (Fig. 3E), despite expression of some inhibitory Ly49 receptors, which recognize MHC I.

Transplantable murine melanoma B16 cell lines are well-established models for studying experimental cancer therapies and NK cell tumor surveillance function (23). Injection of B16 cells into *Rag2^{-/-}Il2rg^{-/-}* mice leads to rapid formation of metastatic foci in the lungs (24). To investigate the tumor-killing ability of the ITNK cells in vivo, we injected 2 million OHT-treated or untreated DP thymocytes from *flx/flx* mice into *Rag2^{-/-}Il2rg^{-/-}* recipients to allow reprogramming of thymocytes to ITNKs in vivo (fig. S7F). Two weeks later, each recipient was injected with B16F10 melanoma cells. Four weeks after the initial thymocyte transplantation, recipients were killed and analyzed. Mice injected with phosphate-buffered saline or with untreated DP cells had ~200 metastatic foci in the lungs. In contrast, mice injected with OHT-treated DP thymocytes had ~20 tumor colonies in the lung (Fig. 3F and fig. S7G). Therefore, ITNKs were potent killers of tumor cells in vivo and prevented cancer progression.

Deletion of *Bcl11b* led to the rapid disappearance of Bcl11b protein (Fig. 4A). Microarray analysis showed that, in OHT-treated *flx/flx* thymocytes, expression of T cell genes such as TCR β and CD3 was already down-regulated within 24 hours (table S3). In another 24 hours, many genes associated with NK cells were expressed (table S3). Bcl11b is proposed to be regulated by Notch signaling in T cell development (25). The *Drosophila* ortholog of Bcl11 genes, CG6530, is shown to be a direct downstream target gene of Notch signaling (26). To confirm this, we identified putative CSL-binding sites

(CGTGGGAA) (27) at the *Bcl11b* locus, which were conserved between mouse and human Bcl11b genes (Fig. 4B) (table S4). A chromatin immunoprecipitation (ChIP) assay performed with a CSL polyclonal antibody pulled down genomic DNA fragments from T cells. Three genomic regions were greatly enriched in the T cell samples when we used the CSL antibody compared with the control (Fig. 4C). The ChIP result thus confirmed that the canonical Notch signaling directly regulated Bcl11b in T cells (fig. S8).

NK cell-based therapies hold promise in cancer treatment. We are now able to reprogram T cells to ITNKs, which can be extensively expanded but are not malignantly transformed. Rather, they effectively killed tumor cells in vitro and eliminated metastatic cells in mice but did not appear to attack normal cells. Therefore, ITNK cells may serve as a new cell source for cancer immunotherapy and other cell-based therapies.

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S8
Tables S1 to S4
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An Early T Cell Lineage Commitment Checkpoint Dependent on the Transcription Factor *Bcl11b*

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The identities of the regulators that mediate commitment of hematopoietic precursors to the T lymphocyte lineage have been unknown. The last stage of T lineage commitment in vivo involves mechanisms to suppress natural killer cell potential, to suppress myeloid and dendritic cell potential, and to silence the stem cell or progenitor cell regulatory functions that initially provide T cell receptor-independent self-renewal capability. The zinc finger transcription factor Bcl11b is T cell-specific in expression among hematopoietic cell types and is first expressed in precursors immediately before T lineage commitment. We found that Bcl11b is necessary for T lineage commitment in mice and is specifically required both to repress natural killer cell-associated genes and to down-regulate a battery of stem cell or progenitor cell genes at the pivotal stage of commitment.

T lymphocyte precursors begin the T lineage differentiation program in the thymus, before they fully lose alternative differenti-

ation potentials inherited from their stem cell precursors (1). Through a sequence of distinct stages coupled with proliferation, termed DN1

to DN3, these cells shed B cell, myeloid cell, dendritic cell, and natural killer (NK) cell options while turning on successive batteries of T cell genes. A necessary, although not sufficient, driver of this process is triggering of Notch1 through interaction with Delta-like (DL) ligands in the thymic microenvironment, and many transcription factors must collaborate with Notch to establish T cell identity. A pivotal stage in the process is the DN2 stage. This is when the first fully committed cells emerge, and their commitment is marked by the concerted, permanent down-regulation of a group of stem cell- or progenitor cell-associated regulatory genes (2). Repression of these genes is crucial for completing normal T lineage specification; at least three of them—*Tal1*, *Lyl1*, and *Spi1* (PU.1)—can each block T cell development or cause T cell leuke-

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mia if sustained beyond this point (3–6). Correct silencing of such genes can be directly implicated in the extinction of certain alternative lineage potentials [for a review, see (7–9)] and probably also underlies the loss of broader stem cell properties. A major challenge for the field has been to identify the rate-limiting regulator that triggers this complex of silencing events (10).

We have used the expression patterns of multiple transcription factors in T cell precursors as clues to identify candidates that might provide a rate-limiting function to trigger commitment. Bcl11b is highly unusual both in its T lineage specificity and for its steep onset of expression in the early DN2 stage, just preceding commitment (11, 12). Expression is then sustained throughout T cell development, placing Bcl11b expression at the right places and the right times to contribute to T lineage commitment. Previous reports have shown that loss of Bcl11b blocks development of T cell receptor (TCR) $\alpha\beta$ T cells after commitment, at the first TCR-dependent selection event in the thymus (13, 14); later, conditional deletion of this gene causes impaired survival and profound abnormalities of CD4⁺ CD8⁺ TCR $\alpha\beta$ T cells (15). Genetic evidence also suggests that Bcl11b acts as a tumor suppressor for thymocytes (16, 17). Although abnormalities in precommitment development without Bcl11b have not been described previously, early stages when Bcl11b is normally first expressed were not dissected in detail in those reports, and cohorts of differentiating cells were not followed kinetically. Therefore, to examine the earliest roles Bcl11b might play in T cell development, we deleted Bcl11b from floxed *Bcl11b*^{L2/L2} conditional knockout prethymic precursor cells (18) using retroviral transduction of Cre recombinase (fig. S1). To track their development through the T cell specification process, we then cultured these cells and Cre-transduced wild-type cells in vitro with OP9 stromal cells that express Delta-like 1 (DL1) and cytokines Flt3L (FMS-like tyrosine kinase 3 ligand) and interleukin-7 (IL-7) (19), conditions that induce T cell differentiation in vitro (19) (Fig. 1A).

Definitive T lineage initiation is marked by the appearance of Kit⁺ CD44⁺ CD25⁺ DN2 cells; these normally give rise to Kit^{low} CD44^{low} CD25⁺ DN3 cells after commitment. Both control and Bcl11b-deleted progenitor cells from fetal liver or adult bone marrow generated early Kit^{high} DN2 cells (DN2a) (Fig. 1, B and C). Bcl11b-deleted cells differentiated poorly to DN3 cells, however, arresting instead at DN2 and producing an abnormally large population of CD44⁺ Kit⁺ CD25⁺ DN1-like cells. Both of these types of descendants from Bcl11b-deleted precursors expressed distinctively high amounts of the progenitor cell growth factor receptor Kit, especially when derived from fetal precursors (Fig. 1B). The most striking feature of Bcl11b-deficient cells, however, was that differentiation was blocked despite proliferation at least as strong as in controls (fig. S2, A to D). When Bcl11b-deficient arrested DN2a

cells were purified by fluorescence-activated cell sorting and replated for further differentiation, they consistently generated more DN2 cells and more DN1-like cells for at least another 12 days but did not progress to a later stage (table S1);

under the same conditions, control cells progressed through DN3 stage and beyond (Fig. 1, D and E, and figs. S2E and S3), to the CD4⁺ CD8⁺ stage (fig. S3D). Bcl11b-deficient cells grew as well as controls in reduced concentrations of IL-7,

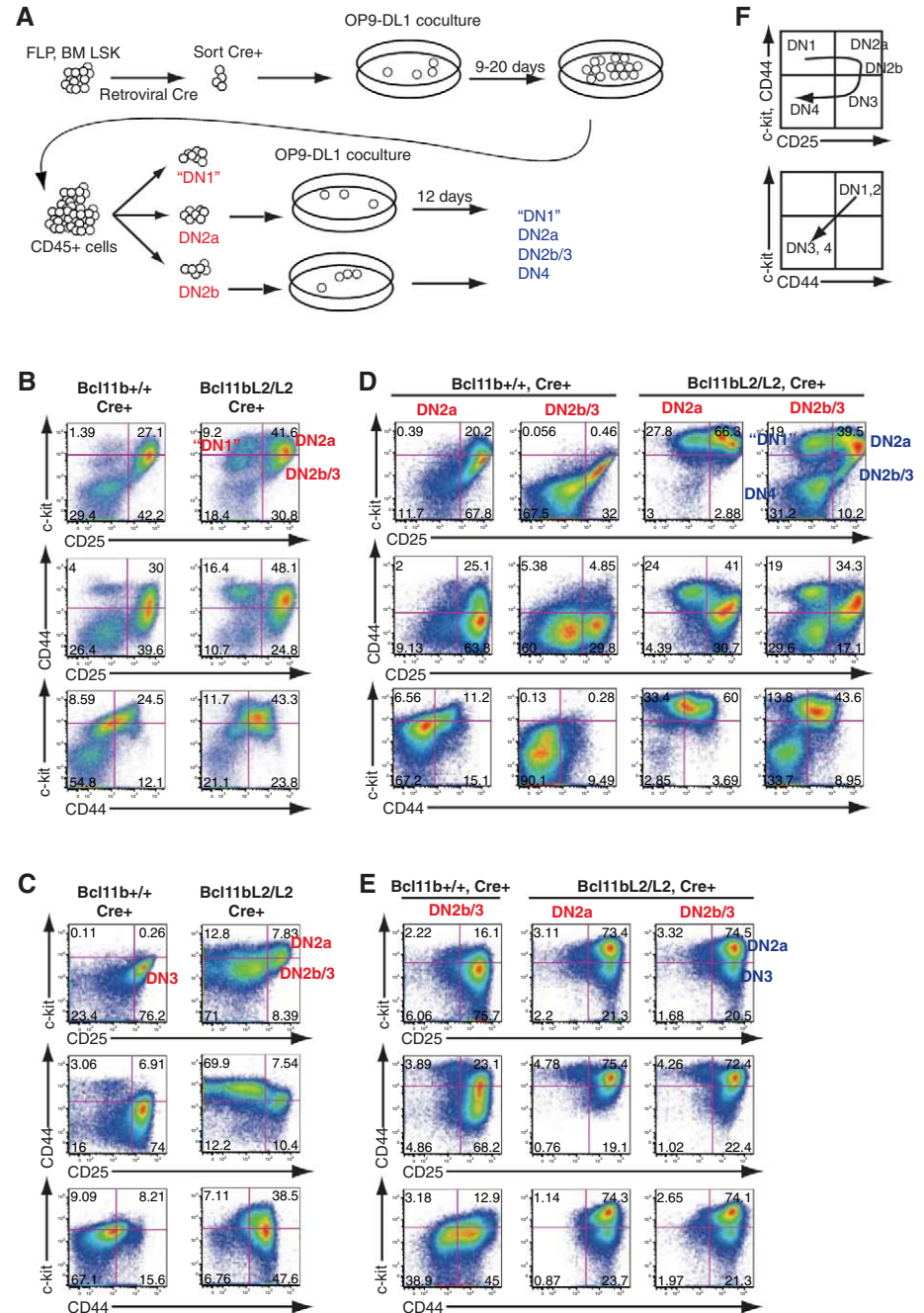


Fig. 1. Generation and developmental arrest of Bcl11b-deficient T cell precursors. **(A)** Experimental scheme. Fetal liver precursors (Lin[−] c-kit⁺ CD27⁺; FLP) from embryonic day 13.5 embryos or bone marrow hematopoietic stem cells (BM LSK) were isolated from Bcl11b^{L2/L2} or wild-type mice, transduced with hNGFR-Cre retrovirus, and sorted 48 hours later and cocultured with OP9-DL1 cells. After 9 days of culture, DN subsets of cells were sorted (red populations). A fraction of each subset was returned to OP9-DL1 coculture for later analysis (blue populations). The remaining cells were analyzed for RNA expression. **(B)** Flow cytometric analysis of transduced fetal liver-derived precursors after 9 days of OP9-DL1 coculture. **(C)** As in (B), for bone marrow-derived precursors after 14 days of OP9-DL1 coculture. **(D)** Flow cytometric analysis of sorted DN2a and DN2b cells from fetal liver precursors after an additional 12 days of OP9-DL1 coculture. **(E)** As in (D), but for sorted DN2a and DN2b cells from bone marrow precursors. **(F)** Schematic of normal differentiation.

but, whereas controls progressed faster to DN3 in these conditions, the *Bcl11b*-deleted cells did not (fig. S2, E and F). Cells that did leak through this block (Fig. 1, B and C; “DN2b” in *Bcl11b*^{L2/L2}) were highly enriched for retention of at least one *Bcl11b* allele (fig. S4). However, loss of *Bcl11b* provided a survival advantage for DN2a and DN1-like cells. In experiments where initial *Bcl11b* deletion was inefficient, cells with a more “advanced” DN2b or DN3 phenotype were generated (Fig. 1B and fig. S4A). After purification and replating, however, these populations yielded “retrograde” DN1 and DN2a descendants (Fig. 1D and table S1) that were highly enriched for complete loss of *Bcl11b* (fig. S4, B and C). The robustness and self-renewal capacity of both DN1-like and DN2 cells without *Bcl11b* contrast with the reported poor viability of later T lineage cells that are deprived of *Bcl11b* (15).

Whereas normal DN2a cells generate few non-T progeny and normal DN2b and DN3 cells are fully committed (2), both DN2a- and DN2b-like cells from *Bcl11b*-deficient precursors generated cells that expressed non-T markers (Fig. 2A). The DN1-like cells generated in cultures of *Bcl11b*-deficient precursors were not early T cell

precursors. Most of them expressed B220 together with CD11c, as well as moderate to high amounts of Kit and substantial Thy1 (Fig. 2A). A substantial fraction of DN1-like cells also expressed the NK cell marker NK1.1 (fig. S5). Cells with these non-T features were generated efficiently from purified *Bcl11b*-deficient DN2 precursors when Notch-DL1 signaling was reduced by transfer to OP9 cells without DL1 (Fig. 2B) or by adding γ -secretase inhibitor (fig. S5), and with the support of Flt3 ligand and IL-7 they expanded in culture on OP9 cells without DL1 (fig. S6).

Although expression of B220, CD11c, and NK1.1 is usually associated with disparate lineages (20–25), gene expression analysis implied many DN1-like cells to be Kit⁺ precursors of interferon-producing killer dendritic cells (IKDCs), proposed to be a subtype of NK cell (20, 21) (Fig. 3). Although they were B220⁺, they expressed no other B cell genes tested (fig. S7; *Cd19*, *Ebf1*). Their modest expression levels of *Mac1* (*Itgam*), *PU.1* (*Spi1*), *SpiB*, and *ROR γ t* (*Rorc*) made them unlikely to be myeloid cells, conventional or plasmacytoid dendritic cells, or lymphoid tissue inducer cells (Fig. 3 and figs. S7 to S9). They

were not precursors because they had lost expression of canonical stem cell or progenitor cell genes (see below). Instead, these “DN1-like” cells expressed high amounts of the NK genes *Id2*, *Il2rb*, *Tbet*, and *Eomes*, along with *Il7r* and *Gata3* expression typical of thymus-derived NK cells (26). The transcription factor genes *Plzf* (*Zbtb16*) and *Nfil3* (*E4bp4*), also implicated in NK cell development (27, 28), were also strongly expressed (Fig. 3 and figs. S7 to S9). Thus, after the DN2 stage, expression of *Bcl11b* may limit T cell access to this NK-like program.

Although access to the NK-like option was most favored under standard OP9 culture conditions, it was not the only one enhanced by loss of *Bcl11b*. Normally, competence to make myeloid progeny is also reduced between the early T cell precursor DN1 stage and DN2a, then extinguished at DN2b (2). Deletion of *Bcl11b* enabled DN2 cells to remain fully competent to generate Mac1⁺ Gr-1⁺ myeloid cells if transferred to myeloid supportive conditions (Fig. 2C). *Bcl11b*-deficient cells therefore retain access to at least two different non-T lineage alternatives.

For normal thymocytes, DN2a is the last stage not only to preserve alternative developmental potentials but also to maintain an extensive complement of stem cell and progenitor cell regulatory gene expression (2). Several highly T cell-specific genes are normally up-regulated at DN2a, including *Bcl11b*, but full T lineage-specific gene expression awaits the DN3 stage (1, 2). The profound shift in regulatory state after the DN2a stage has not been explained by changes in expression of previously studied T lineage transcription factors (10). We therefore asked whether *Bcl11b* might also have a specific role either in triggering T lineage gene expression or in repression of stem cell and progenitor cell genes after the DN2a stage, by comparing gene expression in *Bcl11b*-deficient DN2 cells with that in DN2- and DN3-stage controls (three independent experiments shown in Fig. 3 and figs. S7 to S9).

Bcl11b-deficient precursors began T cell specification in a manner similar to controls, as indicated by the up-regulation of *Cd3e*, *Cd3g*, *Ptcr*, and *Rag1* between the DN1 and DN2a stages in both *Bcl11b*-deficient and control cells (1, 2) (Fig. 3). Expression of these genes was sustained in replated progeny of *Bcl11b*-deficient precursors that retain a DN2a-like phenotype, resembling wild-type DN2a cells (fig. S9). These cells also fully expressed regulatory genes known to be essential for T cell specification (e.g., *Gata3*, *Tcf7*, *Runx1*, *Ikzf1*, *Myb*, *Ets1*, *Tcf2a*, *Tcf12*, the lymphoid growth factor receptor *Il7r*, and Notch targets *Hes1*, *Notch1* itself, and *Myc*, which supports DN3 cell viability) (1, 29). Unlike control cells, *Bcl11b*-deficient DN2 cells in primary culture simultaneously expressed elevated amounts of NK-promoting genes: *Id2*, *Il2rb*, *Nfil3* (*E4bp4*), and *Plzf* (*Zbtb16*) (27, 28). These genes were further up-regulated in progeny that became NK-like, but continued even in cells maintaining a long-term DN2 phenotype (Fig. 3 and figs. S7

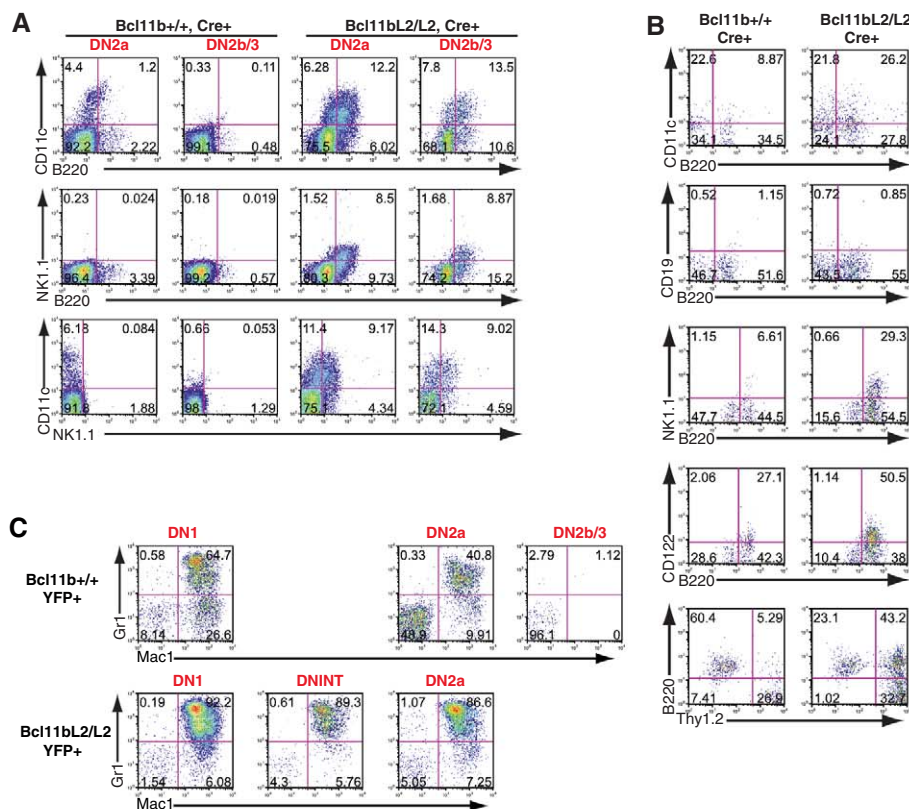


Fig. 2. Non-T lineage differentiation of *Bcl11b*-deleted DN2 and DN1-like cells. (A) Flow cytometric analysis of B220, CD11c, and NK1.1 coexpression on progeny of DN2a and DN2b precursors in OP9-DL1 secondary culture. (B) Flow cytometric analysis of cells derived from DN2 precursors transferred to OP9 control secondary culture. (C) Myeloid developmental competence of T cell precursors from control and *Bcl11b*-deficient precursors. DN1, DN2a, and DN2b cells were generated from bone marrow precursors in primary culture with OP9-DL1 (see fig. S3), sorted after ~20 days, and transferred to OP9 control secondary cultures supplemented with macrophage colony-stimulating factor only. Flow cytometric analyses of Mac1 and Gr-1 expression are shown. Results in each panel are from different independent, single experiments, each representative of three or four experiments.

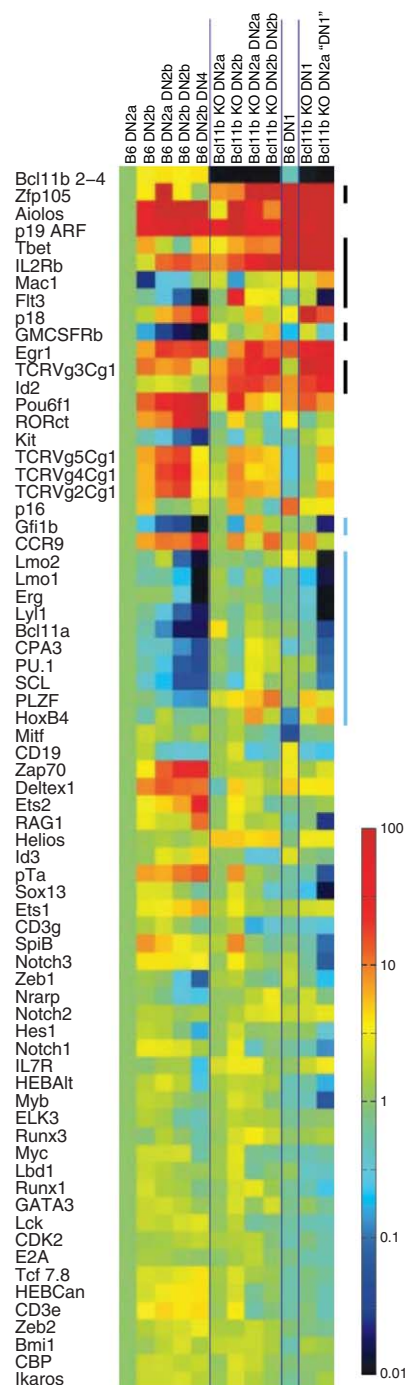


Fig. 3. Gene expression comparison between normal and Bcl11b-deleted cells in early T cell development in vitro. Subsets of control and Bcl11b-deleted cells were sorted both after primary 9-day culture and after secondary culture as indicated. For sorting gates and quantitation of expression levels in fixed units, see fig. S7. “DN4” cells generated by controls in secondary culture may include later stages. Real-time quantitative polymerase chain reaction data are shown as a heat map normalized to levels of each gene in control DN2a cells. Color scale units are log₁₀ for a 10⁴ overall range. Vertical bars on right highlight gene groups of interest. Results are from one of four representative experiments (independent experiments are shown in figs. S8 and S9).

to S9). Thus, loss of Bcl11b may permit early T cell specification to occur in parallel with regulatory “priming” for an innate-like differentiation alternative.

In the context of gene networks that control T cell differentiation, the Bcl11b-deficient DN2a cells also failed in another aspect of repression (10). A “legacy” of progenitor cell regulatory genes (11, 12), including several proto-oncogenes (*Tall*, *Sfpil*, *Lyl1*, *Gfi1b*, *Erg*, and *Bcl11a*), may normally help to sustain proliferation to the DN2a stage. During commitment, the cells normally shift their survival requirements, becoming Notch-dependent, and the progenitor cell genes are repressed in this process (2, 30). Although in vitro T cell differentiation has some abnormal features, all the subsets of cells generated in these cultures from control fetal liver precursors correctly shut off stem cell and progenitor cell genes during secondary culture. When replated, Bcl11b-deficient DN2a cells consistently did not do so (Fig. 3 and figs. S7 and S8). Initially, Bcl11b-deficient cells expressed similar amounts of stem cell and progenitor cell transcription factors as control cells, although often with slightly higher amounts of the progenitor cell cytokine receptor *Flt3*. With sustained culture, Bcl11b-deficient cells continued to resemble the decreasing proportion of control cells that retained a DN1-like or DN2a-like phenotype (fig. S9). However, as the majority of control cells generally progressed to more mature T cell development stages, Bcl11b-deficient cells as a whole diverged from controls. They not only failed to silence but sometimes further up-regulated progenitor cell genes. Alternative lineage markers such as the mast cell gene *Cpa3* and the myeloid growth factor receptor gene *Csf1r2b* were also abnormally sustained. The inability of the cells to differentiate further in the T lineage may thus be explained not by a failure to initiate T cell program activation, but by a failure to mobilize repression, under conditions of Notch and cytokine signaling, if Bcl11b is absent (fig. S10).

Germline Bcl11b knockouts can generate some TCRγδ cells despite a block in TCRαβ development (13). Bcl11b-deficient cells could also generate surface TCRγδ⁺ progeny in vitro (fig. S11). Do these cells not require the same commitment mechanism? Normally, *Rag1* expression is not fully up-regulated until the DN3 stage (1, 2), and this would limit TCRγδ gene rearrangement until after commitment. Even so, DN2a cells can normally generate larger yields of TCRγδ descendants per precursor than DN3 cells (2, 31), which suggests that some TCRγδ emergence may indeed precede mainstream commitment events. Consistent with the strong expression of *Rag1* in Bcl11b-deficient DN2a cells, mutant cells within both DN1 and DN2a samples expressed Vγ-Jγ joined transcripts (Fig. 3 and figs. S7 to S9). Frequently, Bcl11b-deficient cells expressed the fetal-specific Vγ3-Jγ1Cγ1 transcript more than did controls. Fetal-type TCRγδ cells, like NK cells, are programmed to develop in a

unique context of high Id2 expression, which is less permissive for other TCR rearrangements (32). Other “innate” lineages of TCRγδ cells require high levels of *Plzf*, which here are promoted by Bcl11b loss (33). This may indicate that particular TCRγδ sublineages sharing NK-like features may escape an absolute requirement for Bcl11b. Nonetheless, the absolute yield of TCRγδ⁺ cells from any cohort of Bcl11b-deleted precursors was always substantially reduced relative to controls (20 to 30%) (fig. S11). Thus, although in vivo such cells might appear enriched against a more profound TCRαβ deficiency, Bcl11b loss still inhibits the events in T cell development that require passage from DN2a to DN3 stages.

Through dysregulation of progenitor genes, the absence of Bcl11b directly or indirectly disables a crucial component of T lineage-specific commitment machinery. This profound underlying defect may explain why the DN3-like surface phenotype reported for Bcl11b-deficient cells in neonatal germline knockout mice does not confer competence to undergo β-selection (14). The conditions of in vitro culture that reveal the latent self-renewal potential in Bcl11b-deficient DN2a cells undoubtedly sustain greater expansion of DN2-stage cells than the conditions that predominate in the normal thymus. Similar conditions could occur in some intrathymic niches, however, and in these sites loss of Bcl11b could be “rewarded,” lowering a threshold to leukemogenesis. Most important, these results show that Bcl11b’s role distinguishes initial responses of T cell precursors to Notch signaling from a second set of regulatory events that are required for T lineage commitment (fig. S10). Bcl11b may also dissect “adaptive” from “innate” modes of T cell development at an unexpectedly early stage. Our results thus establish this factor as a crucial contributor to both the quality control and the repression of stemness in T cell development.

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Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S11

Tables S1 and S2

References

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An Essential Developmental Checkpoint for Production of the T Cell Lineage

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In early T cell development, progenitors retaining the potential to generate myeloid and natural killer lineages are eventually determined to a specific T cell lineage. The molecular mechanisms that drive this determination step remain unclarified. We show that, when murine hematopoietic progenitors were cultured on immobilized Notch ligand DLL4 protein in the presence of a cocktail of cytokines including interleukin-7, progenitors developing toward T cells were arrested and the arrested cells entered a self-renewal cycle, maintaining non-T lineage potentials. Reduced concentrations of interleukin-7 promoted T cell lineage determination. A similar arrest and self-renewal of progenitors were observed in thymocytes of mice deficient in the transcription factor Bcl11b. Our study thus identifies the earliest checkpoint during T cell development and shows that it is Bcl11b-dependent.

T cells are generated from multipotent hematopoietic stem cells through a series of differentiation steps. The first step in this pathway is the generation of progenitors that have lost erythroid/megakaryocyte potential but retain the capacity to generate other hemopoietic cells, including myeloid, T, and B cells (1–6). We and others have recently identified the next stage, in which the T cell progenitors have lost B cell potential but are still able to generate myeloid cells, dendritic cells (DCs), and natural killer (NK) cells (7, 8). Therefore, the most critical step for development of the T cell lineage is now thought to be at the point where myeloid potential is terminated.

We sought to identify the step at which progenitors become fully committed to the T cell lineage and what regulates this transition. A reliable way to substantiate that a given step is critical for the development of a lineage is to

demonstrate developmental arrest at the stage before that step under particular conditions. In the case of B cell differentiation, deletion of *E2a*, *Ebf*, or *Pax5* genes leads to an early developmental arrest before formation of a functional *IgH* chain gene; these arrested B cell progenitors undergo self-renewal and remain B lineage uncommitted, with the potential to develop along other lineages, including myeloid and T cell (9–11). This case illustrates that such a critical developmental checkpoint exists at the step when uncommitted B cell progenitors become determined to the B cell lineage. Unlike the B cell lineage, to date no such checkpoint has been identified for the T cell lineage before the initiation of *TCR* gene rearrangement.

As T cell progenitors develop, they proceed through developmental stages referred to as DN1 to DN4 (double-negative CD4[−]CD8[−]) that can be tracked by surface phenotype. The DN2 stage can be subdivided into two stages based on transgenic green fluorescent protein (GFP) expression controlled by the proximal *lck* (*plck*) promoter (*lck* is a *src* family kinase selectively expressed by T cells). GFP[−] cells retain non-T lineage potential, including that for myeloid cells, DCs, and NK cells, whereas the latter stage GFP⁺ cells are determined to the T cell lineage (7, 12). We

designate these two stages DN2mt (myeloid-T) and DN2t (T-lineage determined) and term the step between these stages the DN2-determination step. This determination step is thought to be the first critical checkpoint in T cell development (13).

We cultured lineage-negative (Lin[−]) c-kit⁺ Sca-1⁺ (LKS) cells from 13 days post-coitum (dpc) murine fetal liver with immobilized Delta-like 4 (DLL4) protein in the presence of the cytokines SCF (stem cell factor), Flt3L (FMS-like tyrosine kinase ligand), and interleukin (IL)–7 (fig. S1). After 7 days of culture, cells remained at the DN stage (Fig. 1A, left panel), whereas in the control group, where cells were cultured with TSt-4 stromal cells expressing DLL4 (TSt-4/DLL4), generation of CD4⁺CD8⁺ double-positive (DP) cells was observed (fig. S2). Upon closer analysis on DN cells generated in the feeder-free condition, we observed that these cells resembled DN2mt cells (maintained c-kit^{high}CD25⁺) (Fig. 1A, right panel) and thus named them FFDN2 cells (feeder-free-cultured DN2-like cells). By several criteria, the FFDN2 cells appeared identical to DN2mt cells: (i) they gave rise to authentic αβ T cells when transferred to a TSt-4/DLL4 stromal coculture system (fig. S3, A and B); (ii) they retained the potential to produce macrophages (Fig. 1B), NK cells, and DCs (fig. S3, C and D); (iii) intracellular T cell receptor (TCR) β chain protein was not expressed (Fig. 1C); and (iv) their gene expression profiles were similar to those of DN2mt cells (Fig. 1D and fig. S4). Furthermore, GFP expression was not observed in FFDN2 cells generated from progenitors isolated from *plck*-GFP mice (Fig. 1E). It is unlikely that this arrest is due to the failure of TCR gene rearrangement because enforced expression of a functional TCRβ chain gene did not prevent the developmental arrest (fig. S5). FFDN2 cells could not generate B cells (fig. S3E), indicating that dedifferentiation to more primitive progenitors did not occur in this culture system. Of note, FFDN2 cells showed an almost unlimited in vitro expansion (Fig. 1F), while essentially maintaining c-kit and CD25 expression (Fig. 1G) and a developmental potential comparable to that of freshly isolated DN2mt cells (fig. S6). Cells in the c-kit⁺CD25⁺ fraction possessed the potential to maintain long-term culture, because long-term culture could be maintained by using c-kit⁺CD25⁺ cells at the time of passage (fig. S7). Such self-renewal capacity, together with our other results,

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indicated that the DN2-determination step may be a critical checkpoint for T cell development.

To investigate the molecular mechanisms of T cell lineage determination, we searched for an environmental cue that could drive the arrested cells through the DN2-determination step. After

testing various cytokines and Notch ligand conditions in the feeder-free culture system, we found that FFDN2 cells initiate differentiation when the concentration of IL-7 is reduced on day 7 of culture (10 ng/ml to 1 ng/ml). In this induction system, GFP⁺ DN3 cells appear on day 3 after IL-7

reduction (Fig. 2A). These cells did not express myeloid-lineage transcription factors *PU.1* (*Sfp1*) and *C/EBP α* , whereas T cell lineage-associated genes such as *lck*, *Tcf1*, *pT α* , and *Bcl11b* were markedly up-regulated (Fig. 2B). Notably, cells in these cultures developed up to the $\alpha\beta$ TCR-

Fig. 1. Immobilized DLL4 with a combination of cytokines induces self-renewing expansion of immature thymocytes. **(A)** LKS progenitor cells (200 cells) from 13 dpc murine fetal liver were cultured with immobilized Fc-DLL4 supplemented with 10 ng/ml of SCF, IL-7, and Flt3L for 7 days. Generated cells were harvested and stained with the indicated antibodies and analyzed by a flow cytometry. Data are representative of four independent experiments. **(B)** A photomicrograph of macrophages generated from FFDN2 cells. FFDN2 cells induced from green mouse progenitors in a similar manner as (A) were sorted and cultured with Tst-4 stromal cells for 14 days in the presence of 10 ng/ml M-CSF (macrophage colony-stimulating factor). Macrophages are seen as large GFP⁺ cells. Scale bar, 100 μ m. **(C)** Expression of intracellular (ic) TCR β in FFDN2 cells, in negative control cells generated in feeder-free culture using only the Fc portion (Control), and in positive control CD4⁺CD8⁺ DP cells from an adult thymus. **(D)** mRNA expression of lineage-specific genes in cells derived from DN1, DN2mt, DN3, DP, and FFDN2 cells determined by quantitative reverse transcription polymerase chain reaction (RT-PCR). Expression was normalized to acidic ribosomal protein (ARP) mRNA expression, and the mean $\pm$ SD of triplicate samples is shown. Data are representative of three independent experiments. **(E)** Flow cytometric analysis of GFP expression in FFDN2 cells generated from progenitors of plck-GFP mice in comparison with cells generated under Tst-4/DLL4 conditions (Control). Data are representative of three independent experiments. **(F)** A growth curve of FFDN2 cells. Viable cells were enumerated at the indicated time points. **(G)** c-kit versus CD25 expression by FFDN2 cells after long-term culture. Fetal liver LKS cells were cultured under feeder (–) conditions for 30 days and then analyzed by flow cytometry. Data are representative of four independent experiments.

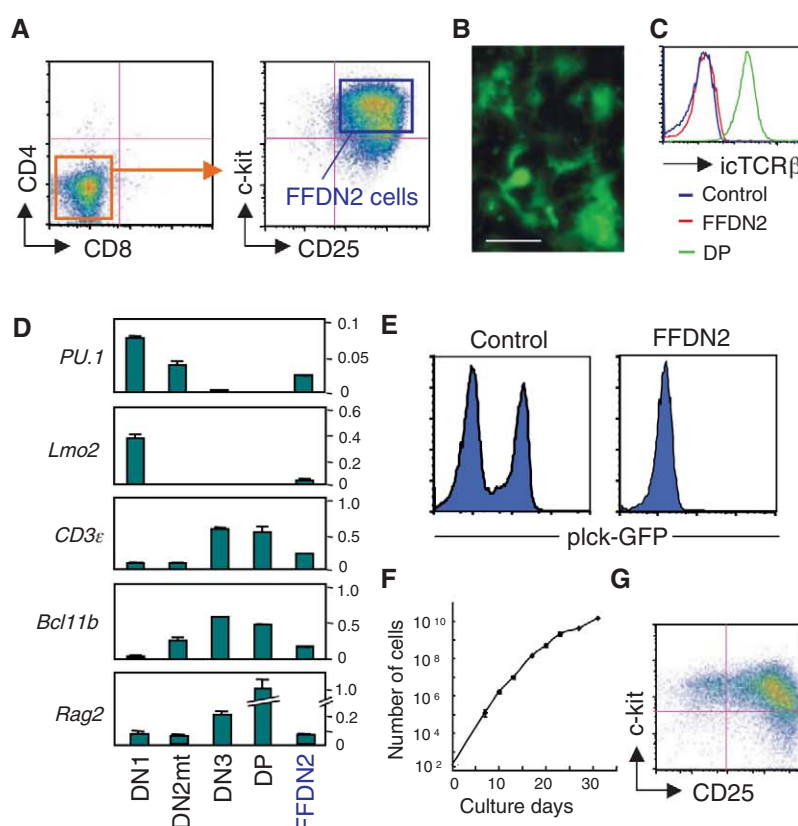
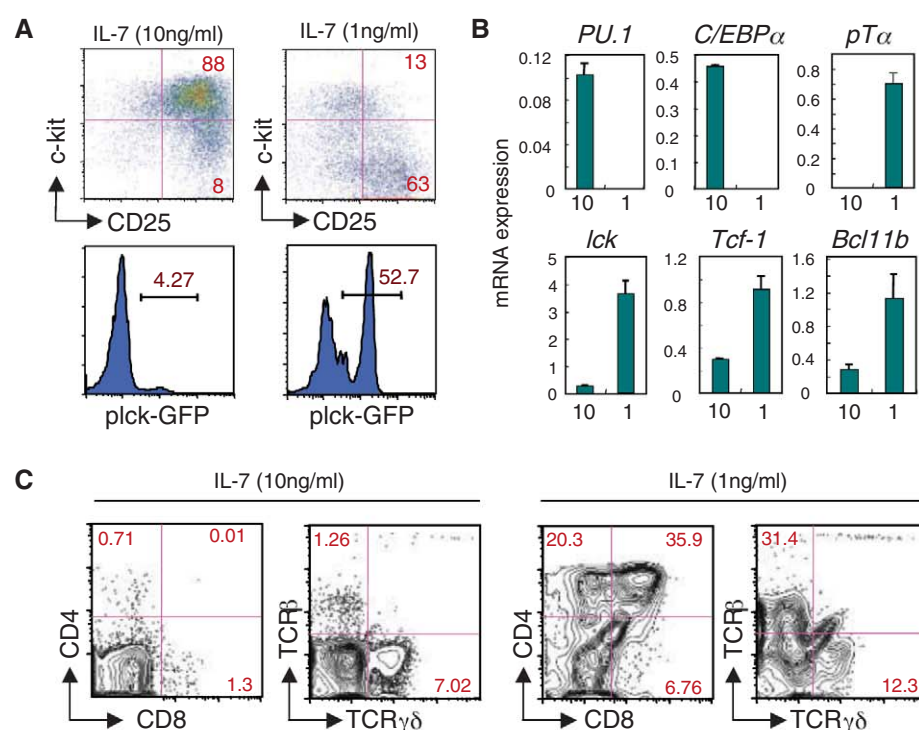


Fig. 2. Reduction of IL-7 concentration induces the generation of DP cells in feeder-free culture. **(A)** LKS cells (200 cells) from 13 dpc fetal liver were cultured with immobilized Fc-DLL4 in the presence of SCF, IL-7, and Flt3L (10 ng/ml). After 7 days, the concentration of IL-7 was maintained or reduced to 1 ng/ml, and the cells were cultured for an additional 3 days. Cells were analyzed by flow cytometry. Data are representative of three independent experiments. **(B)** mRNA expression of lineage-associated genes in cells cultured in the presence of 10 ng/ml or 1 ng/ml of IL-7 in the same manner as (A). Expression was normalized to ARP mRNA expression, and the mean $\pm$ SD of triplicate samples is shown. Data are representative of three independent experiments. **(C)** Flow cytometric analysis of cells generated 7 days after switching to cultures with either high or low IL-7 concentration. Cells were analyzed for the expression of CD4 versus CD8 and TCR β versus TCR $\gamma\delta$. Data are representative of five independent experiments.



expressing CD4⁺CD8⁺ DP stage (Fig. 2C and figs. S8 and S9). Although the kinetics of DP cell growth was delayed compared with that in the TSt-4/DLL4 feeder cell culture system, the final yield of DP cells was nearly identical (fig. S10). The DP cells generated by reducing the concentration of IL-7 appeared to be authentic DP cells, because they give rise to CD4 and CD8 single-positive (SP) cells when transferred to a fetal thymus organ culture

system (fig. S11). These results demonstrated that $\alpha\beta$ TCR⁺ cells can be generated from prethymic progenitors in a “feeder-free” culture system and that the TCR β -selection, which is thought to serve as the critical checkpoint for preTCR formation in progenitors, does not require additional environmental factors in this feeder-free culture system.

Often transcription factors regulate cell lineage determination steps. Among genes up-regulated

by our induction system, we focused on *Bcl11b*, a T cell lineage-specific transcription factor originally identified as a tumor suppressor in murine T cell lymphoma (14). *Bcl11b*-deficient mice exhibit impaired thymocyte development around the DN3 to immature SP stage because of an inability to rearrange the V β to D β gene segments (15). We carefully reexamined the phenotype of fetal thymus cells from *Bcl11b*-deficient mice and found that, at 18 dpc, there was a developmental arrest at the DN2 stage (Fig. 3A). Despite this, the absolute number of DN2 cells was not increased (Fig. 3B), indicating that self-renewing expansion is not so prominent in vivo, a difference that could be due to the limited niche space in the thymus for early progenitors. We cultured these DN2 cells under TSt-4/DLL4 conditions, which can support T cell differentiation up to the DP stage. In such cultures, *Bcl11b*^{-/-} cells continued to proliferate even after 4 weeks, maintaining their DN2 surface phenotype (Fig. 3C). Similar to FFDN2 cells, *Bcl11b*^{-/-} DN2 cells exhibited features of DN2mt cells, including the potential to develop into macrophages and NK cells (Fig. 3D), and loss of B cell potential (fig. S12).

Bcl11b deficiency is lethal around the neonatal period (15). To investigate whether the developmental arrest of *Bcl11b*^{-/-} progenitors is seen in the adult thymus, where T cells are continuously generated, we produced chimeric mice by transferring *Bcl11b*^{-/-} fetal liver cells into irradiated B6Ly5.1 congenic mice. At 8 weeks after transfer, we observed nearly complete developmental arrest at the DN2 stage, with only a few DP cells (Fig. 3E). Similar to ex vivo fetal thymocytes of *Bcl11b*^{-/-} mice and cultured *Bcl11b*^{-/-} DN2 cells, the arrested DN2 cells were equivalent to DN2mt cells. There was no increase in thymic B cells in the recipients of the *Bcl11b*^{-/-} fetal liver cells (fig. S13), indicating that the *Bcl11b*^{-/-} DN2 cells that developed in the thymus did not dedifferentiate into more primitive progenitors in vivo.

The similar stage of arrest in the DLL4/IL-7 cultures and in the *Bcl11b*^{-/-} mice suggested that the arrest in the cultures may be due to a failure to up-regulate *Bcl11b*. To examine this possibility, we retrovirally transduced *Bcl11b* cDNA into fetal liver LKS cells and cultured these transduced cells under DLL4/IL-7 conditions. The *Bcl11b*-transduced cells could give rise to DN3 cells even in the presence of a high concentration of IL-7 (Fig. 4A), and TCR β gene rearrangement was enhanced (Fig. 4B), whereas myeloid-lineage-associated genes were suppressed (Fig. 4C), demonstrating that *Bcl11b* expression eliminated the DN2 arrest that occurred in the DLL4/IL-7 cultures.

As has been reported (16), the absence of *Bcl11b* had a severe impact on the generation of thymic $\alpha\beta$ T cells, whereas there was little effect on the generation of $\gamma\delta$ T cells (fig. S14A). The same is true for cells generated in the DLL4/IL-7 cultures (fig. S14B). These results suggested that the segregation to the $\gamma\delta$ T cell lineage occurs before the DN2mt stage, although the possibility

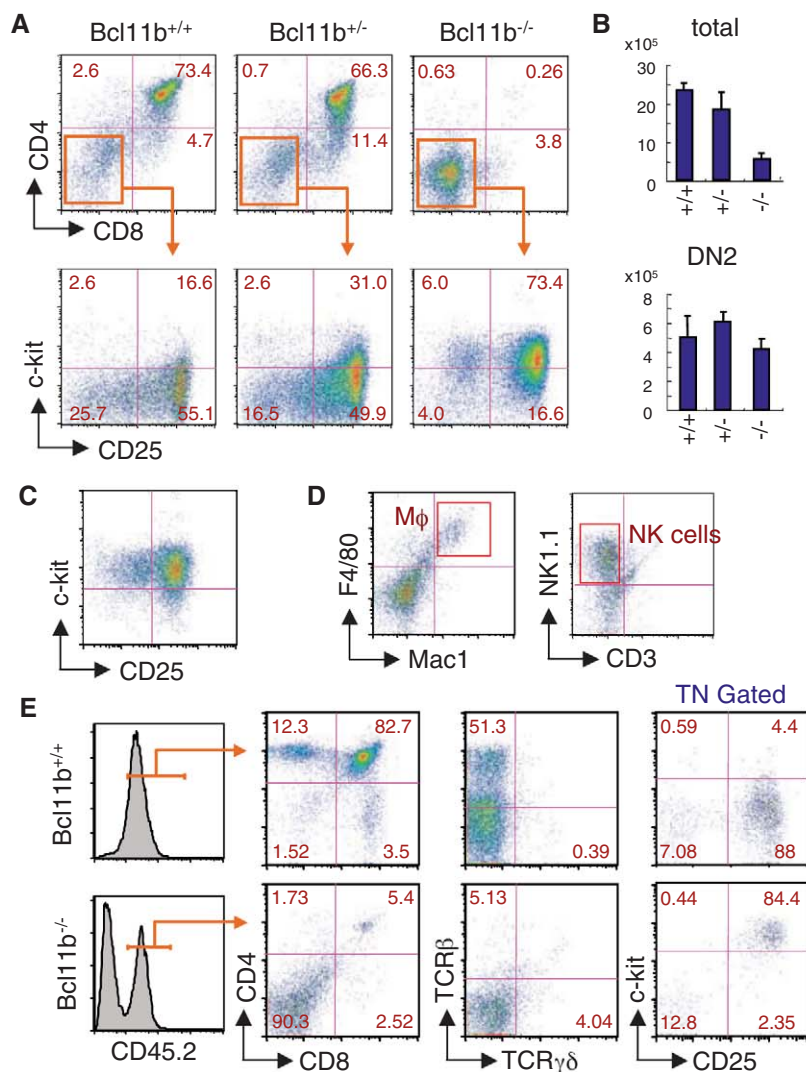
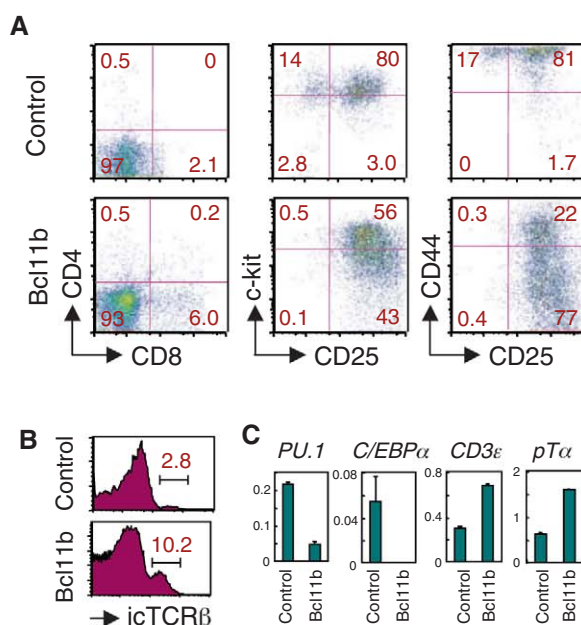


Fig. 3. *Bcl11b* is essential for T cell lineage determination. (A) Flow cytometric analysis of fetal thymocytes from *Bcl11b*^{-/-} mice. Profiles are shown for CD4 versus CD8 of 18 dpc fetal thymocytes, and c-kit versus CD25 of cells gated in upper panels, from the indicated mice. For each group, more than five mice were individually analyzed, and representative profiles are shown. (B) Absolute numbers of total thymocytes and DN2 cells in 18 dpc fetuses of the indicated *Bcl11b* genotypes. More than five mice were individually analyzed for each group, and the mean + SD is shown. (C) Flow cytometric analysis of c-kit versus CD25 of fetal liver LKS cells from *Bcl11b*^{-/-} mice cultured on TSt-4/DLL4 stromal cells for 30 days. Data are representative of three independent experiments. (D) Generation of macrophages and NK cells from cultured *Bcl11b*^{-/-} fetal liver cells. The c-kit⁺ CD25⁺ cells shown in (C) were cultured (200 cells per well) for 7 days with TSt-4 cells in the presence of M-CSF (left panel) or IL-15 (right panel) and analyzed for macrophage and NK cell markers by flow cytometry. Data are representative of three independent experiments. (E) Fetal liver cells from *Bcl11b*^{+/+} or *Bcl11b*^{-/-} mice (Ly5.2) were transferred into lethally irradiated mice (Ly5.1). Flow cytometric profiles of reconstituted thymocytes of recipient mice 8 weeks after transfer are shown. In right panels, profiles of cells gated on CD3⁺CD4⁺CD8⁺ [triple negative (TN)] fraction are shown. For each group, more than five mice were individually analyzed, and representative data are shown.

Fig. 4. Enforced expression of Bcl11b abrogated the DN2 arrest in the DLL4/IL-7 cultures. (A to C) LKS cells from 13 dpc B6 fetal liver were transduced with murine stem cell virus (MSCV)-Bcl11b or MSCV-control vector. Two days later, GFP⁺ cells were sorted and cultured with immobilized Fc-DLL4 in the presence of 10 ng/ml of SCF, IL-7, and Flt3L for 7 days. Flow cytometric profiles of CD4 versus CD8, c-kit versus CD25, and CD44 versus CD25 expression (A), iCTCR β expression (B), and mRNA expression (C) in generated cells are shown. Expression of mRNA was normalized to ARP mRNA expression, and the mean $\pm$ SD of triplicate samples is shown. Data are representative of three independent experiments.



still remains that the $\gamma\delta$ T cells that had been generated from “leaky” DN3 cells underwent compensatory proliferation.

The developmental steps just after the formation of preTCR (DN3 stage) and $\alpha\beta$ TCR (DP stage) serve as critical checkpoints (16, 17), and cells that fail to pass these points succumb to apoptotic cell death. In contrast, the arrested progenitors at the DN2-determination step enter a self-renewal cycle. The appearance of self-renewing progenitors among *Bcl11b*^{-/-} thymocytes may explain the previous findings that loss-of-function mutations in the *Bcl11b* gene are frequently observed in murine T cell lymphomas induced by γ irradiation (14) and that chromosomal aberration disrupting *Bcl11b* gene was identified in human T cell acute lymphoblastic leukemia cases (18), because the acquisition of self-renewal capacity is regarded as the first step in leukemia development. In this context, a similar outcome was recently observed when *Lmo2*,

a known oncogene, was overexpressed in thymocytes and caused the cells to enter a self-renewal cycle in vivo (19).

The present study thus defines a Bcl11b-driven checkpoint at which T cell progenitors terminate non-T-lineage potential in order to become determined to the T cell lineage (fig. S15). Our finding that Bcl11b up-regulation can be triggered by an extrinsic cue, diminished IL-7, suggests that progression through the DN2-determination step is instructed by environmental signals in the thymus. It is quite likely that the reduction in IL-7 signaling is a physiological mediator of this step, because the IL-7R is dramatically down-regulated at the transition from the DN2 to the DN3 stage (20). Considering that Bcl11b is thought to be a transcriptional repressor, we speculate that Bcl11b directly suppresses myeloid-lineage-associated genes, such as *PU.1* or *C/EBP α* , and that such suppression is critical for differentiation toward the T cell fate.

A recent study demonstrated that Bcl11b is expressed in the T cell-like lymphoid cells of lamprey (21). Because a Bcl11b homolog has not been found in animals other than vertebrates (fig. S16), we propose that Bcl11b arose in phylogeny to construct a new lineage distinct from the preexisting innate type killer cells.

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- The authors are grateful to C. Murre, T. Kadesch, Y. Agata, and S. Yamasaki for providing us with reagents and protocols, and to P. Burrows for critical reading of the manuscript. This work was partially supported by Grant-in-Aid for Young Scientists (A) from the Ministry of Education, Science, Sports, and Culture, Japan.

Supporting Online Material

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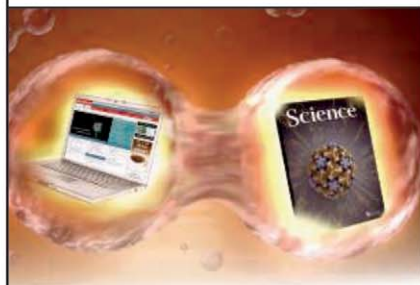
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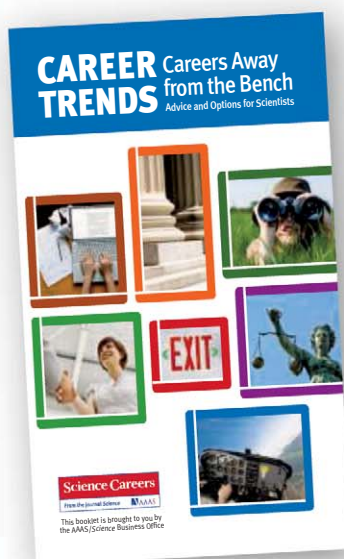
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The selected candidate will be expected to hold a M.D., Ph.D., or equivalent degree. Criteria for selection includes: experience in developing, implementing and/or evaluating behavioral and clinical therapies, specifically in the area of medications development, relevant to alcoholism; experience in managing/leading a complex clinical research organization, experience and expertise in communicating clinical, basic research and programmatic information to scientific and non-scientific audiences; a strong publication record in the field of clinical, behavioral, and medications development research and experience in developing, implementing and managing multidisciplinary and trans-disciplinary research programs on treatments for alcohol use disorders and determinants of post-treatment recovery.

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Applications will be accepted through **September 15, 2010**, or until the position is filled.

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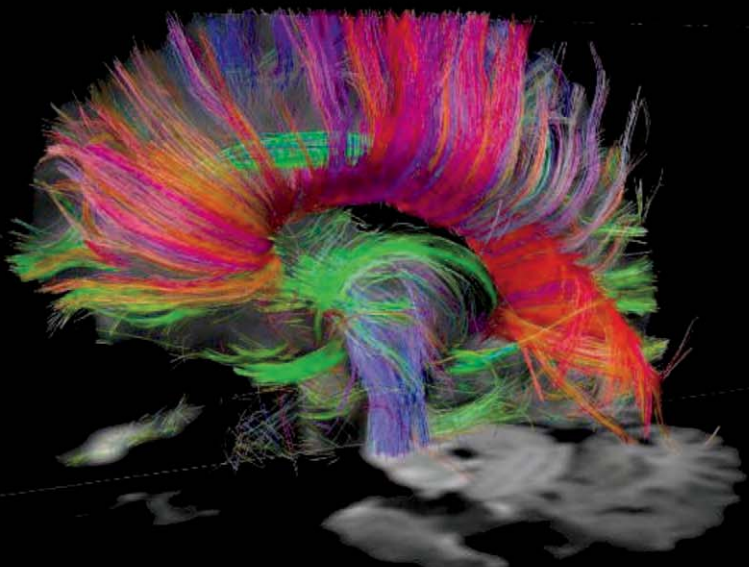
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The Department of Bioinformatics and Computational Biology (DBCBC) at the University of Texas M. D. Anderson Cancer Center (MDACC) is seeking an Associate Director to head its staff of Statistical Analysts and BioAnalysts. The Associate Director will continue the building of a large, high-caliber team of approximately 20 analysts and provide strong intellectual leadership for their collaborative support of preclinical and clinical departments at MDACC. MDACC is strongly committed to growth of the DBCBC in view of the increasingly important role of bioinformatics. A major mission of MDACC is 'personalization' of cancer management through use of molecular biomarkers and biosignatures. As the nation's largest and foremost cancer center, MDACC is uniquely qualified to pursue that mission.

The DBCBC invites applications from outstanding individuals with major expertise and leadership experience in multidisciplinary, collaborative bioinformatics in academia or industry. The successful candidate will have ample opportunity for collaborative publication and grants, but the principal responsibility will be top-quality collaborative support of the various preclinical and clinical programs.

The position requires a Master's degree but the candidate should preferably have a Ph.D. or M.D. in a related field with at least 5 years of management/supervisory experience plus at least 10 years of broad experience in bioinformatics. Strong demonstrated leadership and managerial skills are required. Preferred is a combination of statistical, bioinformatic, computer, and biomedical expertise. Ongoing projects to be designed, analyzed, and interpreted use such technologies as microarrays, mass spectrometry, RNAi, 'NextGen' sequencing, and imaging. The biointerpretive aspects use molecular ontologies, pathways, and molecular structure analysis. The DBCBC (<http://www.mdanderson.org/departments/bioinformatics/>) is intensively involved in large NCI projects including caBIG and TCGA. It is closely allied with the Department of Biostatistics within the Division of Quantitative Sciences.

M. D. Anderson salaries and benefits are very highly competitive. Houston is a cosmopolitan city, featuring fine neighborhoods, excellent private and public schools, superb museums, highly acclaimed opera, symphony, ballet, and theater, and first-rate international cuisine.

Review of applications will continue until the position has been filled. Applicants should apply online at http://www.mdanderson.org/about_mda/careers/, inserting "bioinformatics" as keyword and looking for "Assoc Dir, Quant Research". Individuals with less experience may want to apply for Analyst positions at the same location. For questions, e-mail the Search Committee at dqs-stat-job@mdanderson.org.

The University of Texas M. D. Anderson Cancer Center is an Equal Opportunity Employer and does not discriminate on the basis of race, color, national origin, gender, sexual orientation, age, religion, disability, or veteran status except where such distinction is required by law. All positions at M. D. Anderson are security sensitive and subject to examination of criminal history record information. M. D. Anderson Cancer Center is a smoke-free and drug-free environment. Women and minority candidates are encouraged to apply.



DALIAN INSTITUTE OF CHEMICAL PHYSICS,
CHINESE ACADEMY OF SCIENCES

**Lab Director and Division Directors,
Dalian National Laboratory for Clean Energy**

Dalian National Laboratory for Clean Energy (DNL), based mainly on Dalian Institute of Chemical Physics (DICP), Chinese Academy of Sciences (CAS), is looking for outstanding candidates for DNL director and division directors. The divisions are optimized utilization of fossil energy, low carbon catalysis & engineering, energy saving & energy environment, fuel cell & energy storage, hydrogen energy, biomass energy, solar energy, maritime renewable energy and basic & strategic studies on energy. More details about DICP and DNL can be found at <http://www.dicp.ac.cn>.

The DNL director is responsible for the overall management of the DNL laboratory. He/she provides leadership and direction to DNL, which supports research and education in all divisions and related fields. The division director assesses needs and trends in research in individual division, implements overall strategic planning and policy-setting for division activities.

Successful candidates for these positions should have Ph.D. degree or equivalent experience; a minimum of 10 years experience in energy related researches; substantial research contributions and strong evidence of scholarship as evidenced in publications; proven experience demonstrating innovative leadership in research administration; demonstrated knowledge of the research activities and issues associated with the academic community; knowledge of grant and contract administration, fiscal management, and budget preparation with experience in scientific research support.

DNL offers a highly competitive salary, excellent benefits and a generous scientific research fund.

Applicants may send a complete CV with a full publication list to Dr. Hua'an Zhang (zhangha@dicp.ac.cn) or Prof. Can Li (canli@dicp.ac.cn), or mailed or delivered to the following address: Dalian Institute of Chemical Physics, 457 Zhongshan Road, 116023, Dalian, China.

**Lamont-Doherty Earth Observatory
COLUMBIA UNIVERSITY | EARTH INSTITUTE**

**Faculty Position in Biogeoscience in
the Department of Earth & Environmental Sciences and
the Lamont-Doherty Earth Observatory of Columbia University**

The Department of Earth and Environmental Sciences seeks applicants for a faculty position in biogeoscience, at the tenure track or tenured level. We seek applicants engaged in process-oriented research who will bring crucial new skills, such as use of molecular-level tools, innovative remote sensing techniques, new insight or methodology for understanding biogeochemical cycles, specialist knowledge of ecosystem energetics, and/or application of nano-scale techniques. Our ongoing research in fields related to biogeoscience includes study of biogeochemistry and geochemistry, paleoecology, ecophysiology, climate and paleoclimate, oceanography and paleoceanography, geologic carbon capture and storage, fluid-rock interaction, and the human dimensions of environmental change. Preference will be given to strong applicants who can integrate their work within this spectrum.

Minimum requirements for the position are demonstrated scientific creativity, specialist knowledge in both biology and geoscience, a Ph.D. in a biogeoscience-related field, and capability to teach at the undergraduate and graduate level. Application review will commence immediately and continue until the position is filled. For more information and to apply for this position please visit our online site at:

<https://academicjobs.columbia.edu/applicants/Central?quickFind=53131>

Questions can be addressed to Peter Kelemen (peterk@LDEO.columbia.edu), Chair of the Search Committee.

Columbia University is an Equal Opportunity/Affirmative Action employer.



TEXAS TECH UNIVERSITY
HEALTH SCIENCES CENTER
Paul L. Foster School of Medicine™

Director of Genomics Core Laboratory

Texas Tech University Health Sciences Center in El Paso, Texas seeks a Director for its Genomics Core Facility. This is a full faculty position at the level of Assistant Professor, requiring a strong background in biochemistry and genetics, with hands-on experience in automated DNA sequencing and microarray analysis. Strong opportunities exist for intellectual collaborations with medical research faculty. Next-generation sequencing equipment includes an ABI SOLiD 3Plus in operation, with planned upgrades to a SOLiD 4+ and an Illumina sequencer on order. A new Illumina Hi-Scan bead microarray platform is available for high density SNP genotyping.

Please send a statement of technical and research interests, and a curriculum vitae with three references to:

Dr. Charles C. Miller III, Ph.D.

Associate Dean for Research

Associate Dean for the Graduate School of Biomedical Sciences

Texas Tech University Health Sciences Center - El Paso

Paul L. Foster School of Medicine

E: charles.miller@ttuhsc.edu

P: 915-783-5237

Additionally, please submit an application for the TTUHSC job site:

URL: <http://jobs.texastech.edu/>

Requisition number: **81623**



THE HONG KONG UNIVERSITY OF
SCIENCE AND TECHNOLOGY

School of Science Head of the Division of Life Science

The School of Science of The Hong Kong University of Science and Technology (HKUST) is currently looking for an outstanding academic to lead the newly established Division of Life Science as its Founding Head.

Opened in October 1991, HKUST is an English medium research-intensive university dedicated to the advancement of learning and scholarship, with special emphasis on postgraduate education, and close collaboration with business and industry. Despite its relatively short history, it has rapidly grown to become a major force on the higher education scene in Hong Kong and the world.

To raise Life Sciences teaching and research at HKUST to a new level of excellence, the Division of Life Science will be established within the School of Science, through the reorganization of its existing Departments of Biochemistry and Biology. The Division is to grow, in terms of academic staff, programs, resources and facilities. It will also create synergies in research and scholarship, by enhancing collaborations within the Division as well as interdisciplinary research with other disciplines.

Reporting to the Dean of Science, the Head of the Division will have overall responsibilities for the Division. He/She is expected to lead the Division, formulate policy and to be responsible for the Division's academic advancement (both teaching and research), recruitment and resource allocation. He/She will also be expected to devise strategies to promote and facilitate collaborative and interdisciplinary research with the physical sciences, engineering and other disciplines.

Applicants should be academics with: an excellent record of scholarship eligible for a senior appointment at professor level in the discipline of life sciences; a long-term vision in leading the Division; experience in leading multi-disciplinary collaborative research programs; proven leadership and managerial effectiveness at an appropriate level in higher education institutions; an appreciation of the breadth and depth of research and educational activities in life sciences; and outstanding communication and interpersonal skills.

Professorial appointment with tenure would be made to the successful candidate. Salary is highly competitive and will be commensurate with qualifications and experience. Generous fringe benefits will also be provided.

Application package, including a curriculum vitae, a vision statement as well as the names, addresses, phone numbers and email addresses of at least three referees should be sent to: **Office of the Dean of Science (Re: Head of the Division of Life Science), The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong** (or by fax: (852) 2358 1464; email: dlssc@ust.hk). Review of applications will begin on 1 September 2010 and will continue until the position is filled.

For further information about HKUST and the School of Science, please visit the following websites:

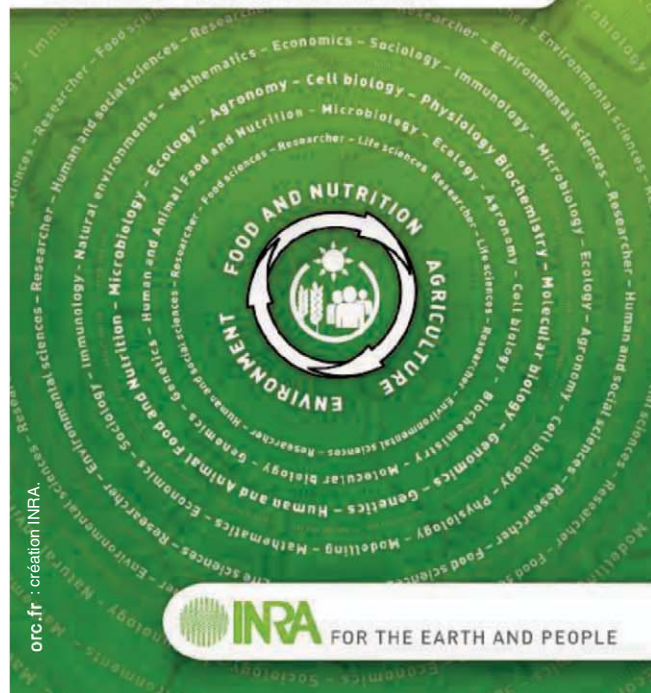
HKUST - <http://www.ust.hk/>; School of Science - <http://science.ust.hk/>

(Information provided by applicants will only be used for recruitment and other employment-related purposes.)

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Applications from 22 June to 2 September 2010

Information : www.international.inra.fr



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FOR THE EARTH AND PEOPLE



Max-Planck-Institut
für Plasmaphysik

The Technische Universität Berlin intends to fill the post of

University professor in Plasma Astrophysics, W2 (Ref. No. II-776),

in its Centre for Astronomy and Astrophysics. The post is a joint appointment (*S-Professur*) within the framework of a co-operation agreement between the Technische Universität Berlin and the Max-Planck-Institut für Plasmaphysik (Garching and Greifswald).

The scientific focus of the appointment lies in the research area of plasma-astrophysics (high temperature plasmas, cold plasmas, relativistic plasmas and interaction with high energy particles, space plasmas, spectroscopy, laboratory astrophysics). It is expected that the successful candidate will represent the discipline in research and be active in acquiring contract funding. He or she will also be involved in the Bachelor and Masters teaching programme of the Centre for Astronomy and Astrophysics. The teaching duties involve five hours a week (5 SWS).

Candidates must fulfill the requirements for university professorship appointments according to § 100 of the Berliner Hochschulgesetz (BerlHG), and should have teaching experience and an outstanding research record in the field of plasma-astrophysics (with expertise in at least one or several of the abovementioned research areas).

The Technische Universität Berlin wishes to ensure equal opportunity employment for men and women; it therefore specifically invites women with the above qualifications to apply for this post. Seriously handicapped persons with equal qualifications will be given preference.

Written applications (**quoting the reference number II-776**) with the usual documentation should be sent by August 31, 2010 to the **President of the Technische Universität Berlin, Fakultät II Mathematik und Naturwissenschaften – Geschäftsstelle Physik – Sekretariat EW 2-1, Hardenbergstraße 36, 10623 Berlin.**

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Montpellier, Biomedical Campus Arnaud de Villeneuve Seeking new group leaders

The campus Arnaud de Villeneuve (AdV) supported by CNRS, INSERM and Montpellier University, is devoted to basic biomedical and health research. It is directly connected to the hospital and medical school and is composed of 3 major institutes: the Institute of Human Genetics (IGH), the Institute of Functional Genomics (IGF) and the Center for Structural Biochemistry (CBS). These Institutes use interdisciplinary approaches in the study of a broad spectrum of topics, including **Epigenetics and Genome Dynamics, Development, basic Molecular and Cellular Biology, Neurogenetics, Molecular and Integrated Neurobiology, Oncology, Virology, Molecular Endocrinology and Cardiology, Molecular Pharmacology, Bioinformatics and Structural Biology and Biophysics** as applied to the above research areas.

The scientific environment benefits from the presence of a wide range of state of the art facilities, including Genomics, Proteomics, medium throughput screening, imaging and animal facilities, and high-end instrumentation for structural biology and biophysics.

The AdV campus is seeking to recruit new teams whose research program is related to the above main research topics. Selected group leaders will receive lab space and support from the campus in addition to assistance in the preparation of applications to answer calls for starting grants from French or European agencies. Selection of candidates will be based on international scientific excellence. Particular attention will be given to innovative, high-risk projects. **The closing date for applications is September 10, 2010. Shortlisted applicants will be notified by September 24 and interviewed during October and November 2010. The new groups are expected to start in October 2011.**

For more information, visit the following web site:
<http://campus-AdV.igh.cnrs.fr>

Candidates should send a short CV, 1) a one page description of their main achievements, including a list of selected publications, 2) a short description of their research project, and 3) arrange for three reference letters to be sent separately by e-mail to the address: campus-AdV@igh.cnrs.fr



www.ox.ac.uk/jobs

Professorship of Scientific Visualisation in association with Pembroke College

The University of Oxford intends to make an appointment to the Professorship of Scientific Visualisation in the Oxford e-Research Centre (OeRC) from 1 October 2011 or earlier, if possible.

The Professor will be expected to establish an internationally-leading research group in scientific visualisation. The overarching research objectives of the new chair include new algorithms and techniques for scientific visualisation and the development of visual analysis tools to facilitate understanding of increasingly complex and rich scientific data. The post holder is expected to engage with computational and imaging problems in such disciplines as astrophysics, fluid dynamics, oceanography, biomedicine, and material sciences as well as with research groups in the social sciences and humanities.

The Professor of Scientific Visualisation will be based in the OeRC and the opportunity to hold a faculty position in the Computing Laboratory will be offered. The person appointed will hold a non-stipendiary fellowship at Pembroke College. A three-year Postdoctoral Research position has been allocated to this post, funded by the Oxford Centre for Collaborative Applied Mathematics (OCCAM).

The successful candidate will have a substantial international reputation, including an excellent publication record, and will have the ability to lead and motivate a research team and to secure significant research funding. The person appointed will be an effective and committed teacher and be able to contribute to the longer-term development of the subject and to its promotion both within Oxford and externally.

Please see the further particulars at www.admin.ox.ac.uk/fp/ for more details about the post and for full instructions before making an application. Applications, including a covering letter and full CV, and naming three referees should be received no later than Monday 13 September 2010 by Dr Gwen Booth, Personnel Officer, Senior Appointments at professorships@admin.ox.ac.uk If you have a query about how to apply, please contact Mrs Elaine Eastgate at professorships@admin.ox.ac.uk or telephone: +44 (0)1865 280189.

Applications are particularly welcome from women and black and minority ethnic candidates, who are under-represented in academic posts in Oxford.

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Science Careers

From the journal *Science*



Tenure Track Professors (at all levels)

The Pennsylvania State University is embarking on a comprehensive and transformative investment in systems genomics, complex traits and biological variation. This cross-college endeavor coordinates faculty recruitments and team-building efforts to address critical problems in the life sciences affecting agriculture, energy production and healthcare. Development of computational and informatics systems for the analysis of genomic data is an integral part of the strategic plan. The PSU Systems Genomics Initiative is a concerted effort to bring predictive power to our understanding of biological systems and realize the potential of biology-based tools.

We seek to appoint multiple tenure-track professors at all levels from Assistant and Associate Professor through to Full Professors and Endowed Chairs who will further Penn State's leadership role in the fields of Systems Genomics and Bioinformatics. We seek faculty interested in analyzing genomic data, undertaking systems and functional genomics and in applying these results to a broad range of biological problems. These will include Computer and Information Scientists, Social Scientists, Life Scientists, Physicists, Mathematicians, Statisticians and Biomedical researchers. Appointments will be made both at the main campus at University Park in Central Pennsylvania and also in the College of Medicine, located at the Penn State Medical Center in Hershey, PA. We seek interactive faculty who can work across disciplines and in a team to provide novel insights into current and future issues. We anticipate that many candidates will have joint appointments and join one of our centers of excellence in genomics, such as the Center for Comparative Genomics and Bioinformatics (<http://www.bx.psu.edu/>) or Center for Medical Genomics (<http://www.huck.psu.edu/institutes-and-centers/medical-genomics>).

These positions feature outstanding research space and competitive start-up packages and state-of-the-art shared instrumentation for sequencing (<http://www.huck.psu.edu/>) and computational analysis (<http://www.ics.psu.edu/>). Penn State has achieved great successes in the field of genomics and bioinformatics with an impressive collection of publications and analysis software, including Galaxy (<http://galaxy.psu.edu/>), a universal platform that can continuously adapt and gain new capabilities to serve the needs of experimental and computational scientists.

Please submit electronically a cover letter, including future research plans, curriculum vitae and the contact information of three references to Deb Stauffer at dsg3@psu.edu

Review will start in August and continue until multiple positions have been filled.

Penn State is committed to affirmative action, equal opportunity and the diversity of its workforce.

Opportunities as limitless as Penn State.

www.psu.jobs



Sanford

**ASSISTANT SCIENTIST POSITIONS
SANFORD CHILDREN'S HEALTH
RESEARCH CENTER**

Sanford Children's Health Research Center (SCHRC; Sioux Falls) invites applications from researchers for full time faculty positions at the rank of Assistant Professor within Sanford Research/USD and the Department of Pediatrics of the Sanford School of Medicine at The University of South Dakota. A historic \$400 million gift by philanthropist T. Denny Sanford has allowed for expansion of Sanford Research/USD and dynamic development of programs specifically focused on children's health. Sanford Children's Health Research Center (SCHRC) is a two-site campus, with locations in Sioux Falls, SD and La Jolla, CA. The La Jolla site is located within the Sanford-Burnham Institute for Medical Research, allowing for a unique partnership which includes open access to their state of the art core facilities and providing the basis for an integrated, world class, academic pediatric research network.

We seek outstanding scientists with research programs that contribute to molecular understanding and treatment of childhood diseases and developmental disorders. Areas of interest include, but are not limited to: genetics, cell biology, cancer biology, and developmental neuroscience/biology. To augment existing strengths, scientists using various model systems in the study of pediatric disease/developmental disorders or those focused on the study of rare pediatric diseases are encouraged to apply. Successful candidates will have PhD, DVM, MD or MD/PhD degrees and join the energetic and collegial research community at Sanford Research/USD. Candidates will be expected to develop an independent and vigorous extramural research program, complement the interdisciplinary and collaborative nature of the growing SCHRC and promote translational research. The researchers will hold both Sanford Research/USD and Sanford School of Medicine faculty appointments.

Significant institutional support including modern laboratory space and state-of-the-art facilities will be provided in the new Sanford Research Center. In addition, a comprehensive compensation package will be tailored to the individual's qualifications.

Candidates should submit a detailed curriculum vitae, description of research experience and future plans, and at least three letters of recommendation. Application materials should be sent to:

David A Pearce Ph.D.
Director, Sanford Children's Health Research Center
Sanford Research/USD
Professor, Department of Pediatrics
Sanford School of Medicine of
The University of South Dakota
2301 East 60th Street North
Sioux Falls, SD 57104-0589
Telephone: 605-312-6004
FAX: 605-312-6071

Email: pearced@sanfordhealth.org
<http://www.sanfordhealth.org/Research/ResearchCenters/SanfordChildrensHealth/>

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POSITIONS OPEN

SENIOR FACULTY POSITION

The Center for Environmentally Beneficial Catalysis and the Department of Chemical and Petroleum Engineering, the University of Kansas

The Center for Environmentally Beneficial Catalysis (CEBC) is seeking an outstanding candidate at the **DISTINGUISHED PROFESSOR** rank as a Kansas Bioscience Authority (KBA) Eminent Scholar as part of the Kansas Alliance for Bioenergy and Biorefining (KABB), a KBA Center of Innovation. The successful candidate should preferably have an international reputation and contribute to CEBC research and education missions ([website: http://www.cebc.ku.edu](http://www.cebc.ku.edu)), and is expected to provide leading expertise in areas including the design, synthesis, and fundamental characterization of metal-based catalysts tailored for specific applications. A detailed position description and application procedures can be found at the University of Kansas [website: https://jobs.ku.edu](https://jobs.ku.edu), search for position **00003860**. Review of applications begins August 15, 2010.

For additional information on required qualifications for an appointment as a Distinguished Professor, please refer to [website: http://www.provost.ku.edu/policy/faculty/dp_guidelines.shtml](http://www.provost.ku.edu/policy/faculty/dp_guidelines.shtml).

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PANCREATIC DEVELOPMENT AND DIABETES

Postdoctoral positions are available immediately in the laboratories of **Drs. Roland Stein** and **Alvin Powers** in the Department of Molecular Physiology and Biophysics and the Vanderbilt Diabetes Center ([website: http://www.mc.vanderbilt.edu/diabetes/drct/](http://www.mc.vanderbilt.edu/diabetes/drct/)). This is an exciting opportunity to conduct cutting-edge research on how islet-enriched transcription factors impact insulin-secreting beta-cell function or proliferation activity under normal and diabetic conditions. The postdoctoral fellows will work in an outstanding scientific environment with state-of-the-art research facilities and frequent interactions with other scientists. Candidates should have expertise in developmental biology or molecular physiology and expertise in basic molecular biology. Send curriculum vitae, description of previous research experience(s), as well as names and e-mail address of three references electronically to: **e-mail: roland.stein@vanderbilt.edu** and/or **e-mail: al.powers@vanderbilt.edu**.

Vanderbilt University is committed to principles of equal opportunity.

POSTDOCTORAL POSITIONS

Postdoctoral position to model landscape change in fire-dependent communities and link habitat model with existing model for red-cockaded woodpecker populations. Twenty-one-month position starting January 2011. The position is through Virginia Tech, but will involve extensive travel to work with other research groups. Home location is negotiable. We seek an individual with interest and expertise in modeling, forest/plant ecology, conservation, and/or avian population biology. Interested candidates should submit curriculum vitae and statement of interest upon applying electronically at [website: http://www.jobs.vt.edu](http://www.jobs.vt.edu) (posting #0100361). Three letters of recommendation should be electronically sent to **Dr. Jeff Walters**, e-mail: **jrwalt@vt.edu**. Review of applications will begin September 1, 2010. *Virginia Tech is an Equal Opportunity/Affirmative Action Institution. Individuals with disabilities desiring accommodations in the application process should notify the search chair by the review begin date.*

CAREER OPPORTUNITY. Doctor of Optometry (O.D.) degree in 27 months for Ph.D.s in science and M.D.s. Excellent career opportunities for O.D.-Ph.D.s and O.D.-M.D.s in research, education, industry, and clinical practice. This unique program starts in March of each year, features small classes, and has 12 months devoted to clinical care.

Contact the **Admissions Office**, telephone: **800-824-5526** at The New England College of Optometry, 424 Beacon Street, Boston, MA 02115. Additional information at [website: http://www.neco.edu](http://www.neco.edu). Email: **admissions@neco.edu**.

POSITIONS OPEN

The College of Sciences and Mathematics at Auburn University located in Auburn, Alabama ([website: http://www.auburn.edu/cosam](http://www.auburn.edu/cosam)), is seeking candidates for the position of **POSTDOCTORAL FELLOW** in the sciences and mathematics. From time to time, postdoctoral positions become available under a variety of research grants and projects in the college. We are seeking applications from individuals with a Ph.D. in any one area such as biology, chemistry, geology, geography, mathematics, statistics, physics, or related fields. The candidate selected for these positions must be able to meet eligibility requirements to work in the United States at the time appointment is scheduled to begin and continue working legally for the proposed term of employment; excellent communication skills required. The positions are available for a minimum of one year (full time, 12 month) with renewal possible, based on performance and funding. Salary will be commensurate with education and experience. Review of applications will begin after July 5, 2010, and continue throughout the year as positions become available. Please send curriculum vitae and statement of research interests along with a list of three references and contact information to: **Ms. Stephanie Woodley, COSAM Human Resources Generalist, 315 Roosevelt Concourse, Auburn University, AL 36849**. E-mail: **woodlsc@auburn.edu**.

Minorities and women are encouraged to apply. Auburn University is an Affirmative Action/Equal Opportunity Employer.

HEALTH SCIENCE SENIOR RESEARCH SPECIALIST

Posting #0806477

The University of New Mexico (UNM), Brain Imaging Center seeks a Health Science Senior Research Specialist in the broad area of nuclear magnetic resonance imaging (MRI) of small animals, such as mice and rats. Candidates for this position must have B.A. or B.S. in a biomedical specialty and thorough knowledge of nuclear spin physics. The ideal candidate will have several years of MRI experience on Bruker MRI systems, using Paravision 5.0. The documented ability to perform diffusion tensor imaging, arterial spin labeling, chemical shift imaging, and functional MRI would greatly enhance the candidate's suitability. Experience with small animals is also needed. To apply: For complete information including closing dates, minimum requirements, and instructions on how to apply for this or any UNM position please visit our [website: http://UNMJobs.unm.edu](http://UNMJobs.unm.edu), or telephone: **505-277-6947**, or visit our human resources service center at **1700 Lomas Boulevard N.E., Suite 1400, Albuquerque, NM 87131**. *Equal Employment Opportunity/Affirmative Action.*

POSTDOCTORAL SCIENTIST

The Department of Biochemistry at George Washington University (GW) seeks a highly motivated and creative individual to advance his/her career in cancer biology. The position will study the contribution of signaling components in DNA damage response and cell cycle progression in breast cancer cells, as well as gain novel insights about functional interactions between coregulatory proteins and histones in normal and cancer cells. The laboratory is interested in signaling-dependent posttranslational modifications, protein-DNA interactions, transcriptional controls, and secreted proteins.

Qualifications: Ph.D. in biochemistry, molecular biology, or cell biology. Prior experience in the needed laboratory methods and clear career goals are highly desired. Excellent scientific writing ability and strong oral communication skills are a must. Individuals with prior research publications as well as strong laboratory skills are highly desired.

To apply for this position visit our [website: http://www.gwu.jobs](http://www.gwu.jobs) and search for posting number **0601920**. *GW is an Equal Opportunity Employer/Affirmative Action Employer.*

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